2 Inflammation

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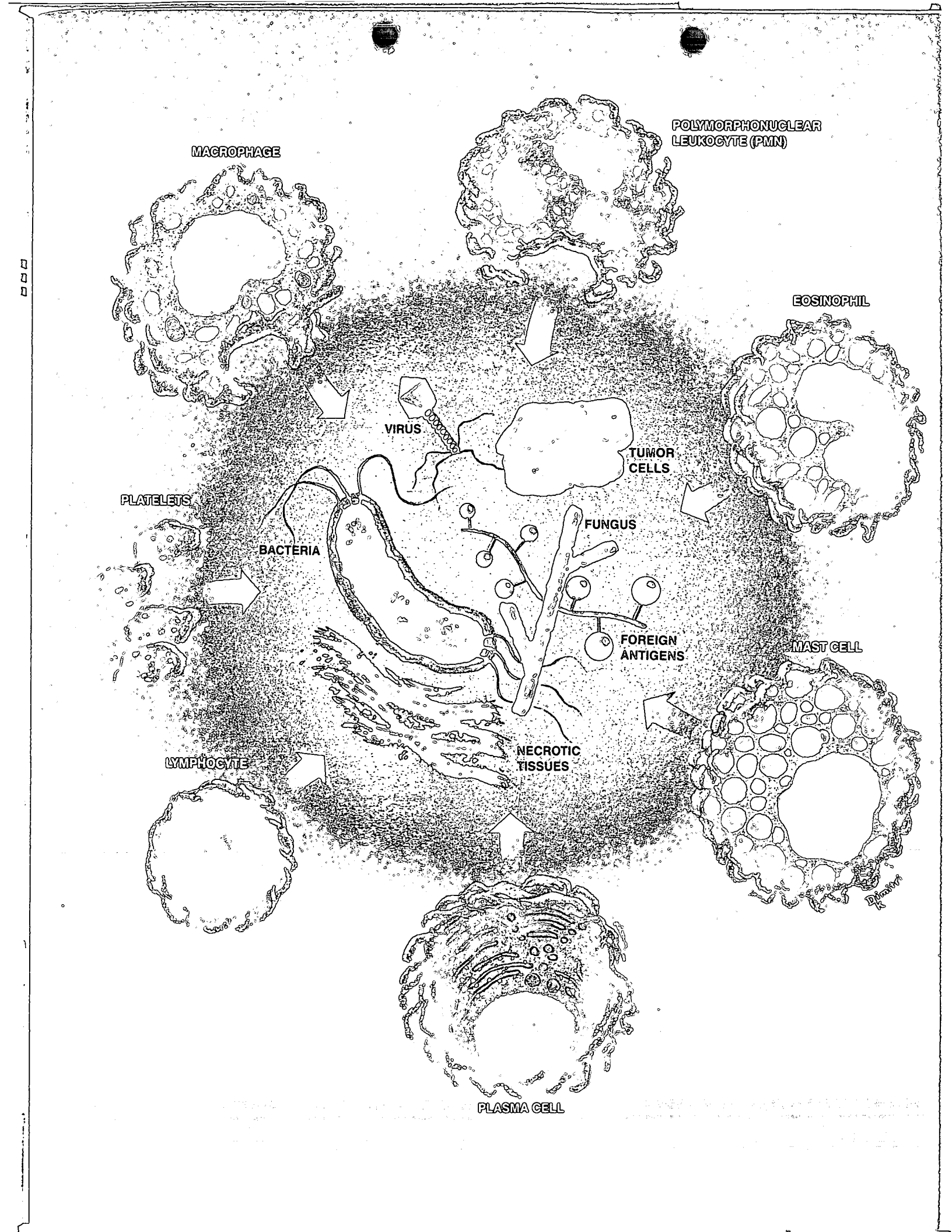
Figure 2-1. Participants in acute and chronic inflammatory reactions.


MACROPHAGE
POLYMORPHONUCLEAR LEUKOCYTE (PMN)
EOSINOPHIL
VIRUS
TUMOR CELLS
PLATELETS
FUNGUS
BACTERIA
FOREIGN ANTIGENS
MAST CELL
NECROTIC TISSUES
LYMPHOCYTE
PLASMA CELL

Inflammation is a reaction of the microcirculation characterized by movement of fluid and white blood cells from the blood into extravascular tissues. This is frequently an expression of the host's attempt to localize and eliminate metabolically altered cells, foreign particles, microorganisms, or antigens. The clinical signs of inflammation were described in Classical times; the Greeks and Romans noted the association of redness (rubor), heat (calor), swelling (tumor), and pain (dolor) with acute injury to tissues. These are the clinical signs with which we are most familiar and with which we associate response to injury.

Under normal conditions the inflammatory response eliminates the pathogenic insult and removes injured tissue components. This process accomplishes either regeneration of the normal tissue architecture and return of physiologic function or the formation of scar tissue to replace what cannot be repaired. Further extension of injury or the effects of the inflammatory response itself may lead to loss of function of the organ or tissue. The mechanisms responsible for the localization and clearance of foreign substances and injured tissues are initiated by the recognition that injury to tissues has occurred. This is followed by an amplification phase of the inflammatory response, in which both soluble mediators and cellular inflammatory systems are activated. After generation of inflammatory agents and elimination of the foreign agent, inflammatory responses are terminated by specific inhibitors of the mediators. Under certain conditions the ability to clear injured tissue and foreign agents is impaired, or the regulatory mechanisms of the inflammatory response are altered. In these circumstances inflammation is harmful to the host and leads to excessive tissue destruction and injury. In other instances, an immune response to residual microbial products or to altered tissue components also triggers a persistent inflammatory reaction.

Initiation of the inflammatory response following tissue injury occurs within the microvasculature at the level of the capillary and postcapillary venule. Within this vascular network are the major components of the inflammatory response, including plasma, platelets, red blood cells, and circulating white blood cells (Figs. 2-1 and 2-2). Normally these components are confined within the intravascular compartment by a continuous layer of endothelium that is connected by tight junctions and separated from the tissue by a limiting basement membrane. Following injury to a tissue, changes occur in the structure of the vascular wall, leading to a loss of endothelial cell integrity, leakage of fluid and plasma components from the intravascular compartment, and emigration of both red and white blood cells from the intraluminal space into the extravascular tissue.

Specific inflammatory mediators produced at the sites of injury (Fig. 2-3) regulate the response of the vasculature to injury. Among these mediators are vasoactive molecules that act directly on the vasculature to increase vascular permeability. In addition, chemotactic factors are generated that recruit white blood cells from the vascular compartment into the injured tissue. Once present in tissues, recruited white blood cells secrete additional inflammatory mediators that either enhance or inhibit the inflammatory response.

Historically, inflammation has been referred to as either **acute or chronic inflammation,** depending on the persistence of the injury, its clinical symptomatology, and the nature of the inflammatory response. **The hallmarks of acute inflammation include accumulation of fluid and plasma components in the affected tissue, intravascular stimulation of platelets, and the presence of polymorphonuclear leukocytes** (Fig. 2-4). **By contrast, the characteristic cell components of chronic inflammation are macrophages, lymphocytes, and plasma cells** (Fig. 2-5).

Activation of the inflammatory response results in one of three distinct outcomes. Under ideal conditions the source of the tissue injury is eliminated, the inflammatory response resolves, and normal tissue architecture and physiologic function are restored. In some cases, however, the nature of the acute inflammatory reaction is such that the area is walled off by the collection of inflammatory cells, a process that results in destruction of the tissue by products of the polymorphonuclear leukocytes (also known as neutrophils). This is the mechanism by which an **abscess** is formed. Alternatively, if the tissue is irreversibly injured despite elimination of the initial pathologic insult, the affected tissue's normal architecture is often replaced by scar. The third possibility is that the inflammatory cells may fail to eliminate the pathologic insult, in which case the inflammatory reaction persists. The area of chronic inflammation often expands, leading to fibrosis and scar formation.

Vascular Permeability

Alterations in the anatomy and function of the microvasculature are among the earliest responses to tissue injury (Fig. 2-6). An early vascular response to mild injury of the skin involves a transient vasoconstriction of arterioles at the site of injury. This

vasoconstriction is mediated by both neurogenic and chemical mediator systems, and usually resolves within seconds to minutes. **Vasodilation** of precapillary arterioles follows, with an increase in blood flow to the tissue. **This vasodilation is caused by the release of specific mediators and is responsible, in part, for the redness and warmth at sites of tissue injury.**

In conjunction with the vasodilation and increased blood flow, alterations in the permeability of the endothelial cell barrier result in increased leakage of fluid from the intravascular compartment into extravascular spaces. If not effectively cleared by lymphatics, fluid accumulates in the extravascular space. A net increase in extravascular fluid is called **edema**; its clinical manifestation is swelling. The loss of fluid from the intravascular compartment as blood passes through the capillary venules leads to local stasis and plugging of dilated small vessels with red blood cells. These changes are reversible following mild injury, and within several minutes to hours the extravascular fluid is cleared through lymphatics. The endothelial injury is reversed and the normal structure of the microcirculation is reestablished.

The pathologic changes described above are characteristic of the classic "triple response" first described by Sir Thomas Lewis. In the original experiments, a dull red line developed at the site of mild trauma to the skin, followed by the development of a red halo (flare) and associated swelling (wheal). Lewis postulated the presence of a vasoactive mediator that causes vasodilatation and increased vascular permeability at the site of injury.

Injury to the vasculature is a dynamic event and frequently involves sequential physiologic and pathologic changes. **Vasoactive mediators**, originating from both plasma and cellular sources, are generated at sites of tissue injury by a variety of mechanisms (Fig. 2-7). These mediators bind to specific receptors on vascular endothelial and smooth muscle cells, causing vasoconstriction or vasodilatation. Vasoconstriction of arterioles decreases blood flow to a tissue; arteriolar vasodilatation increases blood flow and can exacerbate fluid leakage into the tissue. In contrast, vasoconstriction of venules increases the hydrostatic pressure in the capillary bed, potentiating edema formation. Vasodilatation of venules decreases capillary hydrostatic pressure and inhibits the movement of fluid into the extravascular spaces. Therefore, when the role of a particular vasoactive mediator in the development of inflammatory response is being examined, the effects of this mediator on specific tissues and components of the vasculature must be identified.

Binding of vasoactive mediators to endothelial cells results in a complex series of biochemical events causing endothelial cell contraction and gap formation. This break in the endothelial barrier leads to an extravasation (leakage) of intravascular fluids into the extravascular space. **The postcapillary venule is the primary site at which the vasoactive mediators induce endothelial changes.** Endothelial retraction and gap formation is a reversible process. Local injection of classic vasoactive mediators into the skin results in an acute change in vascular permeability that peaks between 15 and 20 minutes after injection. Vascular integrity is restored within an hour. In contrast, direct injury to the endothelium, such as that caused by burns or caustic chemicals, may result in irreversible damage. In such cases the endothelium is separated from the basement membrane, an effect that leads to cell blebbing, that is, the appearance of blisters or bubbles between the endothelium and the basement membrane, and areas of denuded basement membrane. Mild, direct injury to the endothelium may result in a biphasic response: an early change in permeability occurs 15 to 30 minutes after the injury, followed by a second increase in vascular permeability after 3 to 5 hours. When damage is severe the exudation of intravascular fluid into the extravascular compartment increases progressively, reaching a peak between 3 and 4 hours after injury.

Accumulation of fluid within the extravascular compartment and interstitial tissues is referred to as **edema**; excess fluid in the cavities of the body is labeled an **effusion.** Edema fluid with a low protein content (specific gravity of <1.0) is called a **transudate.** Edema fluid with a high protein concentration (specific gravity >1.0) is termed an **exudate**; it is frequently characterized by a high lipid content and cellular debris. Exudates are observed early in acute inflammatory reactions and are produced by mild injuries, such as sunburn or traumatic blisters. When exudates or effusions occur in tissues in the absence of a prominent cellular response, they are termed **serous.** A serous fluid usually has a yellow, strawlike color, but when red blood cells are present the fluid has a red tinge and is referred to as **serosanguineous.** Under conditions that activate the coagulation system, large amounts of fibrin may be deposited in tissues, a process that results in a **fibrinous** exudate. An inflammatory exudate or effusion that contains prominent cellular components is described as **purulent. Purulent exudates and effusions are frequently identified with pathologic conditions such as pyogenic bacterial infections, in which the predominant cell type is the polymorphonuclear leukocyte.**

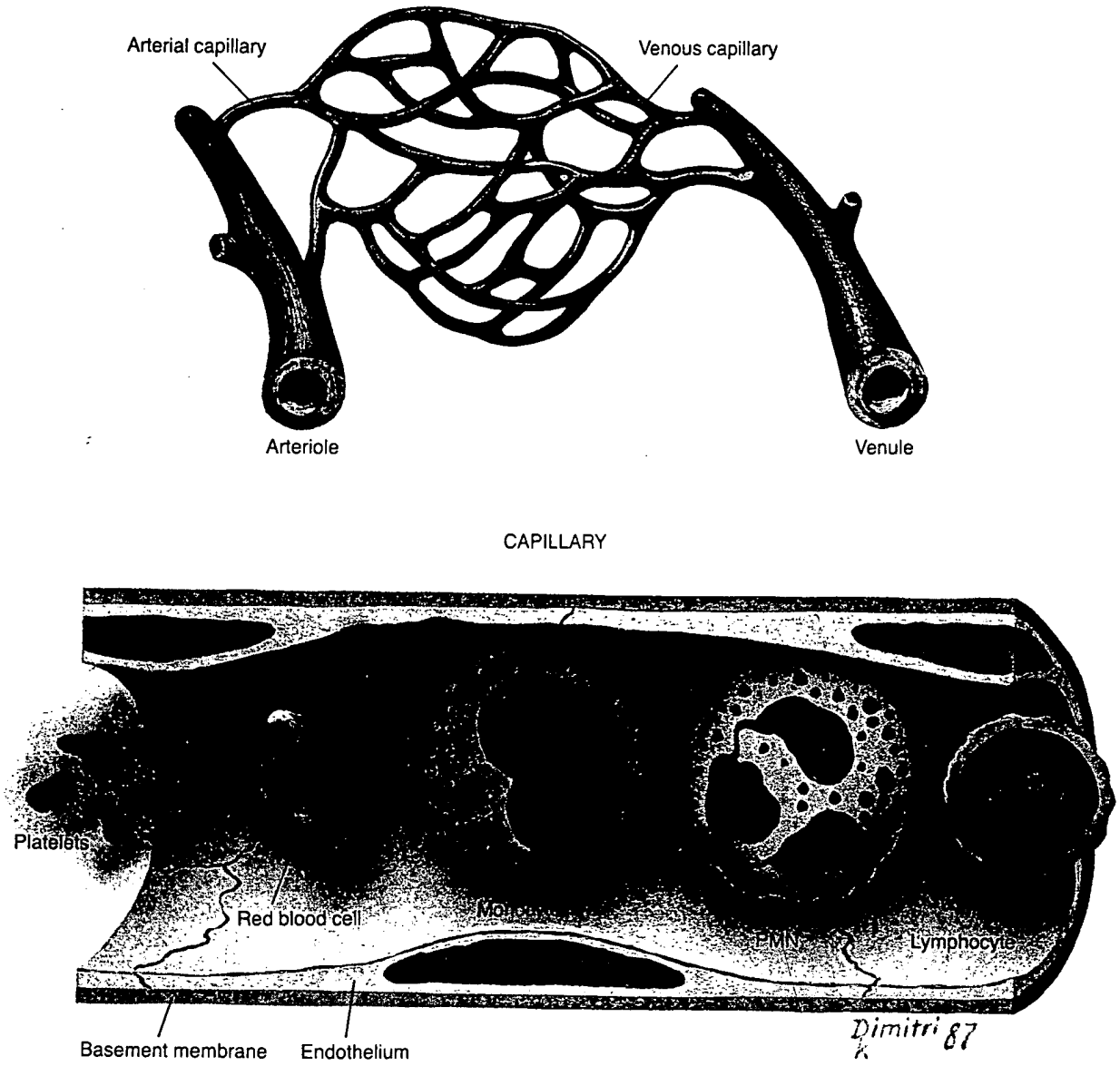


Figure 2-2. The microcirculation and cellular components of the blood.

Sources of Vasoactive Mediators

The primary sources of vasoactive mediators are cells and plasma. Important cellular sources of vasoactive mediators are circulating platelets, tissue mast cells, and basophils.

Platelets

The platelet plays a primary role in normal homeostasis and in the initiation and regulation of clot formation. It is also an important source of inflammatory mediators, including potent vasoactive substances and growth factors that modulate mesenchymal cell proliferation (Fig. 2-8). The platelet is a cell approximately 2 μm in diameter, lacking a nucleus but containing at least three distinct kinds of granules: dense granules rich in serotonin, histamine, Ca^{2+}, and adenosine diphosphate (ADP); α-granules containing fibrinogen, coagulation proteins, platelet-derived growth factor (PDGF), and other peptides and proteins; and lysosomes containing acid hydrolases.

When platelets come in contact with fibrillar collagen (following vascular injury that exposes the interstitial matrix proteins) or thrombin (following

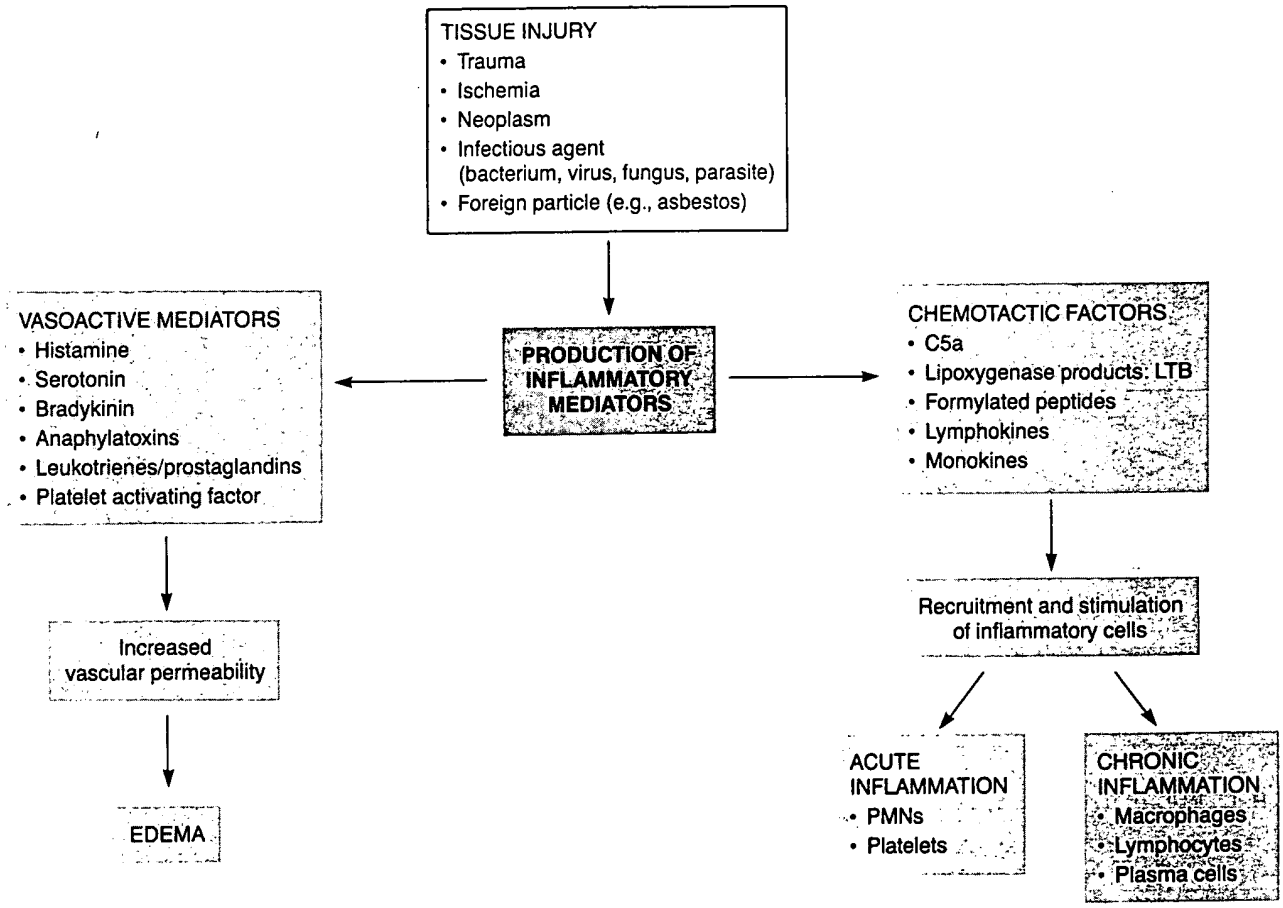


Figure 2-3. Mediators of the inflammatory response.

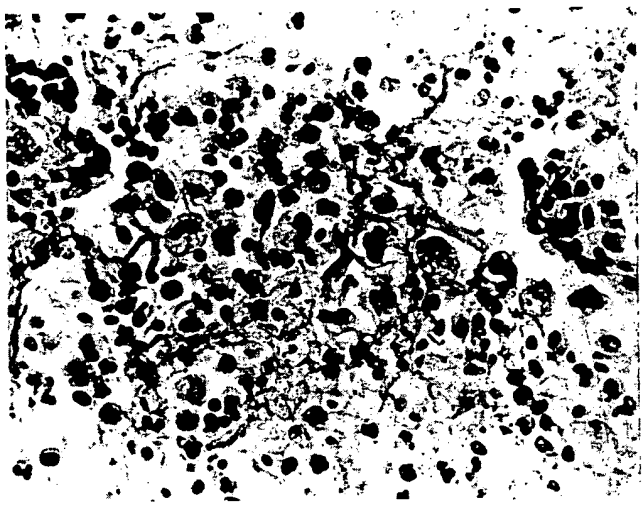


Figure 2-4. Acute inflammation. Interstitial edema and numerous polymorphonuclear leukocytes are present.

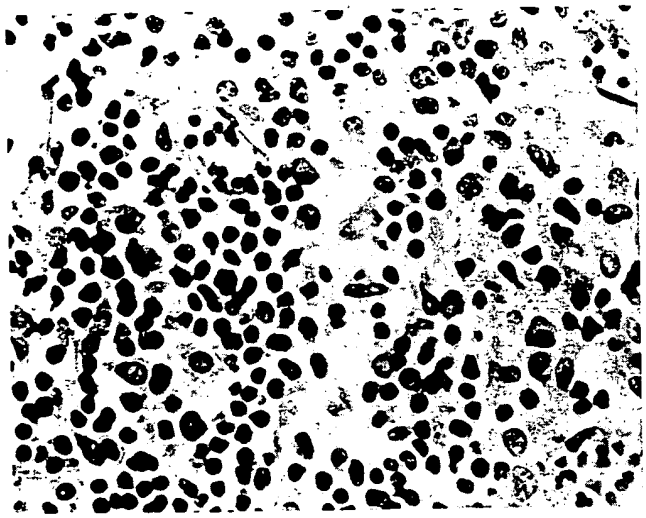


Figure 2-5. Chronic inflammation. Macrophages, lymphocytes and plasma cells predominate.

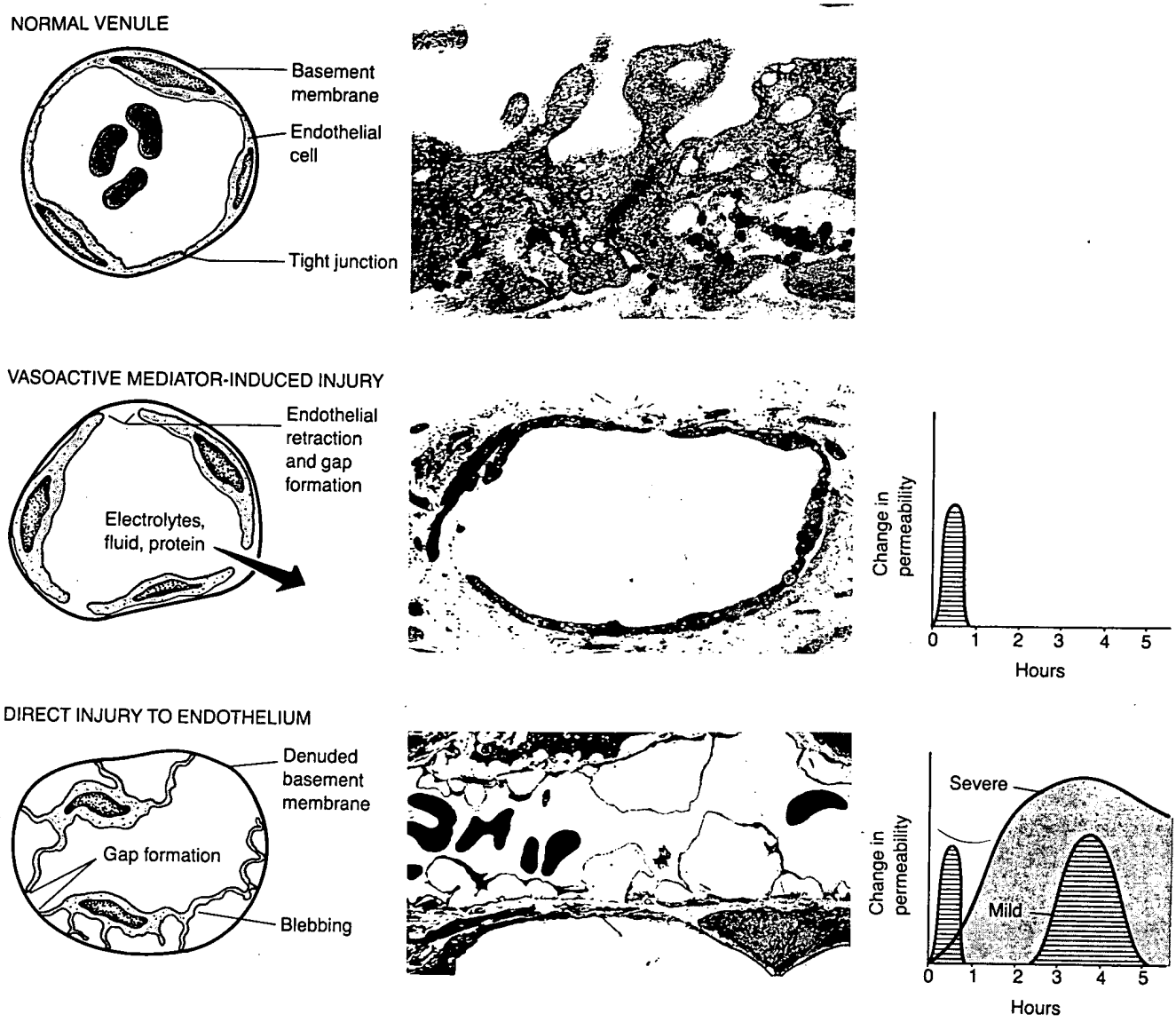


Figure 2-6. Response of the microvasculature to injury. The wall of the normal venule is sealed by tight junctions between adjacent endothelial cells. During mild injury, the endothelial cells separate and permit the passage of the fluid constituents of the blood. With severe direct injury, the endothelial cells form blebs and separate from the underlying basement membrane. Areas of denuded basement membrane allow a prolonged escape of fluid elements from the microvasculature.

activation of the coagulation system), platelet adherence, aggregation, and degranulation may occur. Degranulation is associated with the release of **serotonin (5-hydroxytryptamine) and histamine**, mediators that directly induce changes in vascular permeability. In addition, the arachidonic acid metabolite **thromboxane A_2** is produced. Thromboxane A_2 not only plays a key role in the second wave of platelet aggregation, but also possesses smooth muscle constrictive properties.

Mast Cells and Basophils

The mast cell and basophil are additional cellular sources of vasoactive mediators. When antigen binds to IgE immunoglobulin and cross-links these molecules on basophil and mast cell surfaces, secretory release of these mediators from electron-dense cytoplasmic granules into extracellular tissues occurs (Fig. 2-9). These granules contain histamine, acid mucopolysaccharides (including heparin), and chemotactic

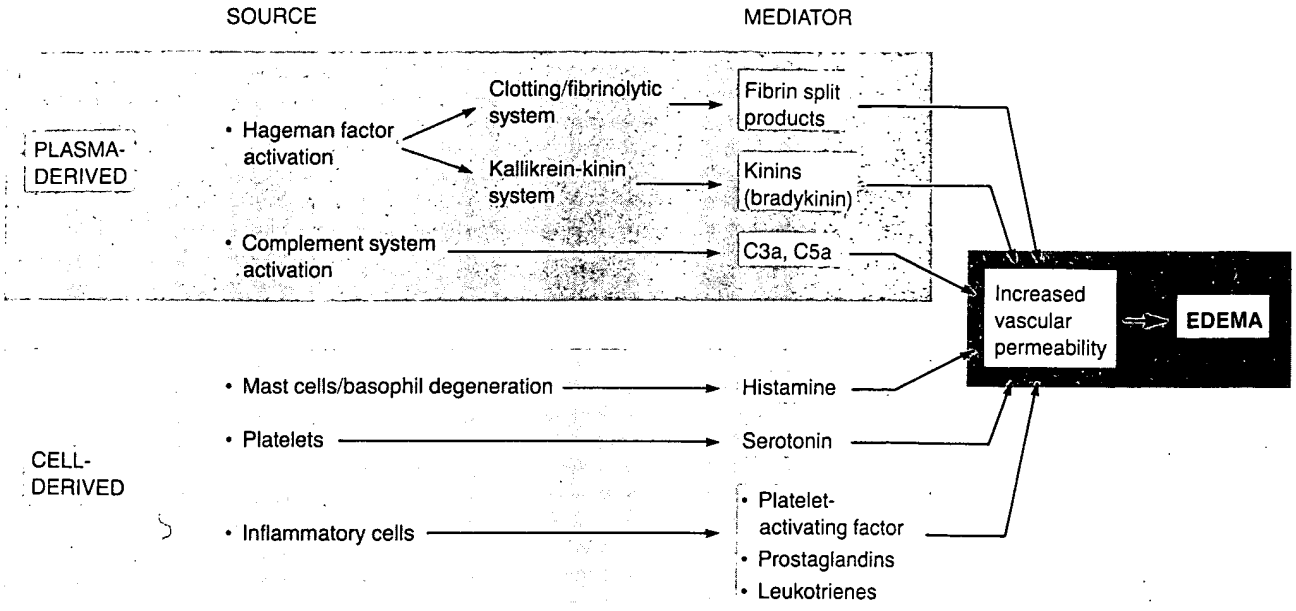
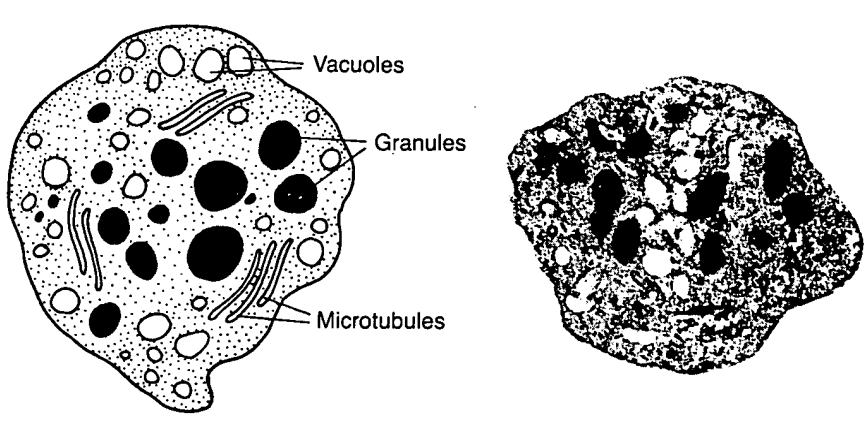


Figure 2-7. Vasoactive mediators of increased vascular permeability.

mediators for neutrophils and eosinophils. Because of their ability to secrete specific mediators following stimulation, both mast cells and basophils play an important role in the regulation of vascular permeability and bronchial smooth muscle tone, especially in many forms of allergic hypersensitivity reactions (see Chapter 4). Histamine is released from the electron-dense granules when IgE-sensitized cells are stimulated with antigen or the anaphylatoxins derived from the third and fifth components of the complement system (C3a and C5a). When injected into skin, both histamine and serotonin induce reversible endothelial cell contraction, gap formation, and edema.

The most important effects of histamine and serotonin occur early in the evolution of inflammatory reactions. The action of histamine on the vasculature is a result of its binding to specific H_1 receptors in the vascular wall, an effect that can be inhibited pharmacologically by H_1-receptor antagonists. Degranulation of mast cells and basophils may also be induced by physical agonists, such as cold and trauma, as well as by cationic proteins derived from platelets and neutrophil lysosomal granules.

Stimulation of mast cells and basophils also leads to the release of products of arachidonic acid metabolism, including the so-called **slow-reacting substances of anaphylaxis (SRS-As). The SRS-As consist**



CHARACTERISTICS AND FUNCTIONS
- Thrombosis; promotes clot formation
- Regulates permeability
- Regulates proliferative response of mesenchymal cells

PRIMARY INFLAMMATORY MEDIATORS
- Dense granules
  - –Serotonin
  - –Ca^{2+}
  - –ADP
- α-granules
  - –Cationic proteins
  - –Fibrinogen and coagulation proteins
  - –Platelet-derived growth factor (PDGF)
- Lysosomes
  - –Acid hydrolases
- Thromboxane A_2

Figure 2-8. Platelets: morphology and functions.

Mast Cell (Basophils)

CHARACTERISTICS AND FUNCTIONS
- Binds IgE molecules
- Contains electron-dense granules

PRIMARY INFLAMMATORY MEDIATORS
- Histamine
- Leukotrienes (LTC_4, LTD_4, LTE_4)
- Platelet activating factor
- Eosinophil chemotactic factors

Figure 2-9. Mast cells: morphology and functions.

of leukotriene C_4 (LTC_4), leukotriene D_4 (LTD_4) and leukotriene E_4 (LTE_4). These lipoxygenase products of arachidonic acid metabolism induce smooth muscle contraction and increase vascular permeability in the skin. They produce their effects by binding to specific receptors on cell membranes and are important in delayed changes in vascular permeability at sites of inflammation.

Platelet Activating Factor

Stimulation of mast cells and leukocytes results in the generation of another class of vasoactive mediators, first characterized as a platelet activating factor (PAF), having the structure of an acetylated lysophospholipid. The biochemical structure of this compound varies with the species of origin. In the rabbit, PAF has been characterized as 1-0-hexadecyl/octyldecyl-2-acetyl-sn-glycero-3-phosphocholine (AGEPC), a compound that has potent biologic effects at nanomolar concentrations. PAF induces platelet aggregation and degranulation at sites of tissue injury and enhances the release of serotonin and histamine, thereby causing changes in vascular permeability. In addition, it enhances arachidonic acid metabolism in neutrophils, an effect associated with increased motility, superoxide production, and degranulation of the polymorphonuclear leukocyte. PAF also has direct effects on the microvasculature, causing vasodilatation and enhancing vascular permeability at sites of tissue injury.

Hageman Factor (Factor XII)

Additional sources of vasoactive mediators are generated within plasma (Fig. 2-10). Activation of Hageman factor (clotting Factor XII) by exposure to negatively charged surfaces, such as basement membrane, proteolytic enzymes, bacterial lipopolysaccharides, and foreign materials (including urate crystals as occur in gout) results in the proteolytic activation of several additional plasma proteins. The list includes conversion of plasminogen to plasmin, conversion of prekallikrein to kallikrein, and activation of the alternative complement pathway.

Plasmin generated by activated Hageman factor induces fibrinolysis. The products of fibrin degradation augment vascular permeability in both the skin and the lung. In addition, plasmin cleaves components of the complement system in an action that generates biologically active products, including the anaphylatoxins C3a and C5a. C3a and C5a increase vascular permeability in the skin both directly and indirectly (e.g., by a mast cell–dependent mechanism).

Plasma kallikrein generated by activated Hageman factor cleaves high-molecular-weight kininogen, thus generating several vasoactive low-molecular-weight peptides, collectively referred to as **kinins** (Fig. 2-11). The best characterized of these vasoactive kinins is **bradykinin**. Bradykinin is a nanopeptide that, when injected into skin, elicits reversible changes of the endothelium that lead to edema. Many kinins are

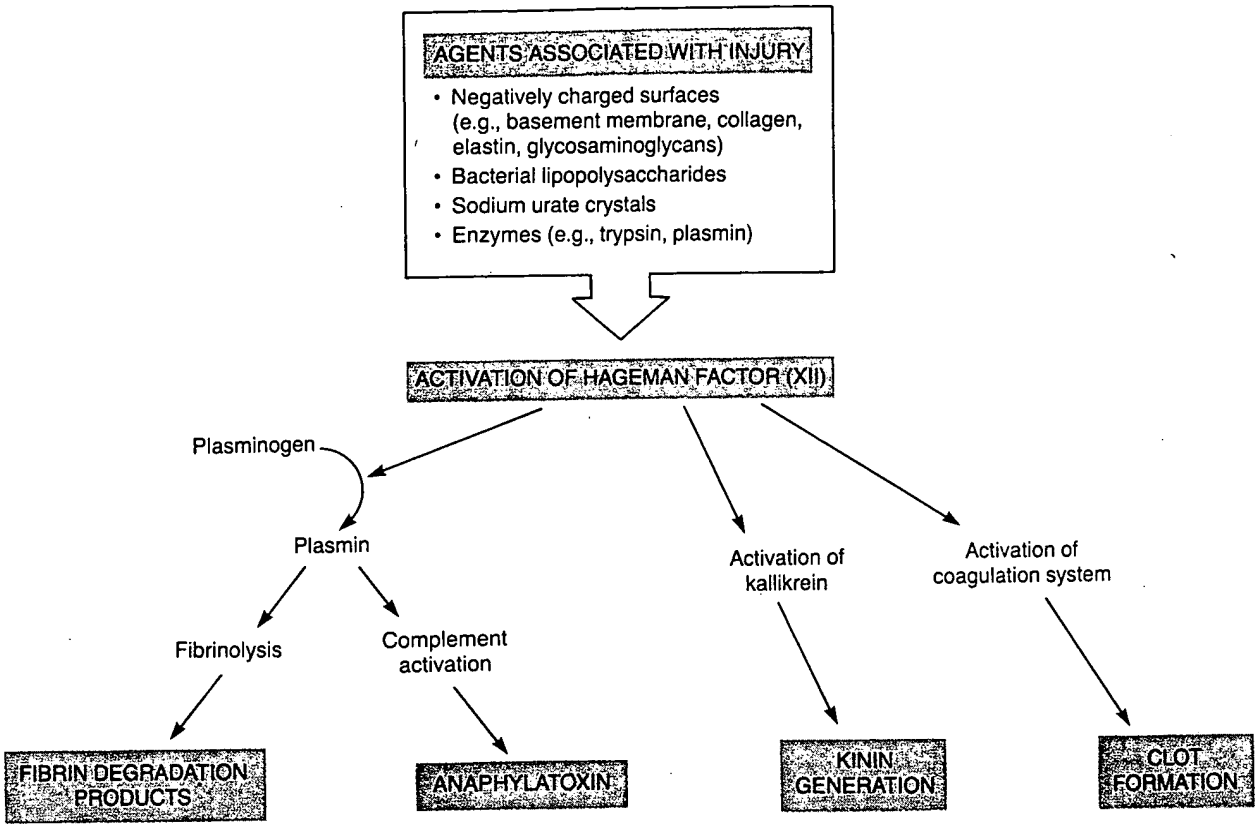


Figure 2-10. Hageman factor activation and inflammatory mediator production.

under the tight regulatory control of specific inactivating enzymes. For instance, two enzymes present in plasma, carboxypeptidase N (kininase I) and a dipeptidase referred to as angiotensin-converting enzyme (kininase II), selectively cleave the carboxy-terminal peptide and dipeptides of bradykinin, respectively. This renders the molecule biologically inactive.

The permeability changes induced by the vasoactive mediators are enhanced by the local production of vasodilative substances. In particular, the vasodilative prostaglandins (PGI_2, PGE_2, and PGD_2) increase edema formation when injected locally at sites of tissue injury. One proposed mechanism for the anti-inflammatory effects of aspirin, indomethacin, and other nonsteroidal anti-inflammatory drugs is their inhibition of prostaglandin production.

Complement System

The c mplement system consists of a group of 20 plasma proteins. In addition to being a source of vasoactive mediators, components of the complement system are an integral part of the immune system and play an important r le in host defense against bacterial infection. Originally described as a biologic effect of serum responsible for the lysis of antibody-coated cells, it is now known that this activity is present in an inactive form in plasma. These proteins are sequentially activated by two independent pathways, termed "classical" and "alternative" (Fig. 2-12).

Classical Pathway

Activators of the classical pathway (Table 2-1) include antigen–antibody immune complexes and products of bacteria and viruses. The activation of the classical pathway involves recognition of the inflammatory agent by the first component of complement, C1. C1 consists of three separate proteins, C1q, C1r, and C1s. When IgM immunoglobulin or molecules of specific IgG subclasses are bound to soluble or fixed antigens on target cells or tissue substrates, alterations in the conformation of the Fc component initiate binding of C1q. This results in sequential enzymatic activation of C1r and C1s. Two additional components of the complement system, C4 and C2, serve as the substrate for the enzymatically active C1s. The action of C1s on C4 and C2 is responsible for the

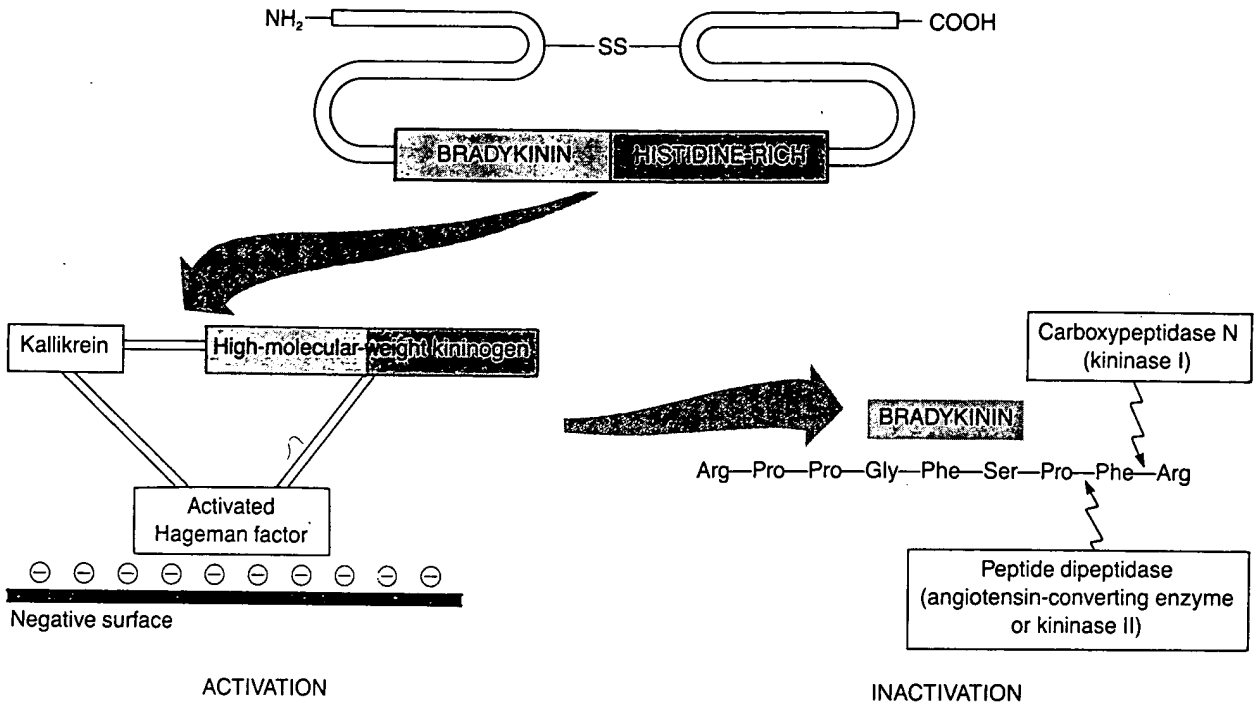


Figure 2-11. The bradykinin precursor, kininogen, interacts with kallikrein and activated Hageman factor to form a trimolecular complex. Kallikrein releases bradykinin from kininogen. Bradykinin is, in turn, inactivated by kininases.

release of the first soluble anaphylatoxin, C4a, and the generation of the complex C4b2a. This complex, in turn, has proteolytic activity for the C3 molecule and has been defined as a C3 convertase. The C3 convertase cleaves C3, generating a second soluble anaphylatoxin molecule, C3a, and a residual product of C3 cleavage, C3b. The resulting multimolecular complex formed with C4b2a binds C5 and initiates hydrolysis of the C5 molecule, a process that generates a third complement-derived anaphylatoxin, C5a, and a residual component of C5 cleavage, C5b. The C5b molecule serves as a nucleus on target cell surface membranes for the sequential binding of C6, C7, and C8, and the polymerization of C9 molecules. This cascade leads to the formation of a macromolecular complex termed the **membrane attack complex.**

The assembly of the membrane attack complex on target cell surfaces occurs through hydrophobic interactions of the molecules. Morphologically, the injury by the membrane attack complex appears as a cylindrical hole in the cell membrane. As a consequence of its highly lipophilic nature, the membrane attack complex alters the phospholipid bilayer and membrane functions, which may ultimately result in the loss of cell membrane integrity, followed by cell lysis. Gram-negative bacteria are protected from the cytolytic action of the membrane attack complex by a peptidylglycan layer. However, lysozyme, an enzyme present in the granules of phagocytic cells, is capable of cleaving the peptidylglycan layer. Once the bacteria are exposed to this enzyme, the membrane attack complex inserts into the cell membrane and lysis is initiated.

Alternative Pathway

Activation of the alternative pathway of the complement system is initiated through derivative products of infectious organisms and through foreign materials (Table 2-1) via a cascade-like interaction of specific plasma proteins. In the alternative pathway, unlike the classical pathway, C1, C4, and C2 are not involved. Activation of the alternative pathway occurs through the binding of C3 with two plasma proteins, factor B and factor D. This results in the formation of an enzymatically active derivative of factor B. The larger fragment, termed Bb, catalyzes the conversion of C3 to C3b and C3a. When C3b is bound to Bb, a C3 convertase is generated, thus greatly amplifying subsequent conversion of C3 and generating additional C3b and C3a. In addition, C5 convertase is formed, which in turn generates C5b (soluble C5a), and the membrane attack complex is subsequently

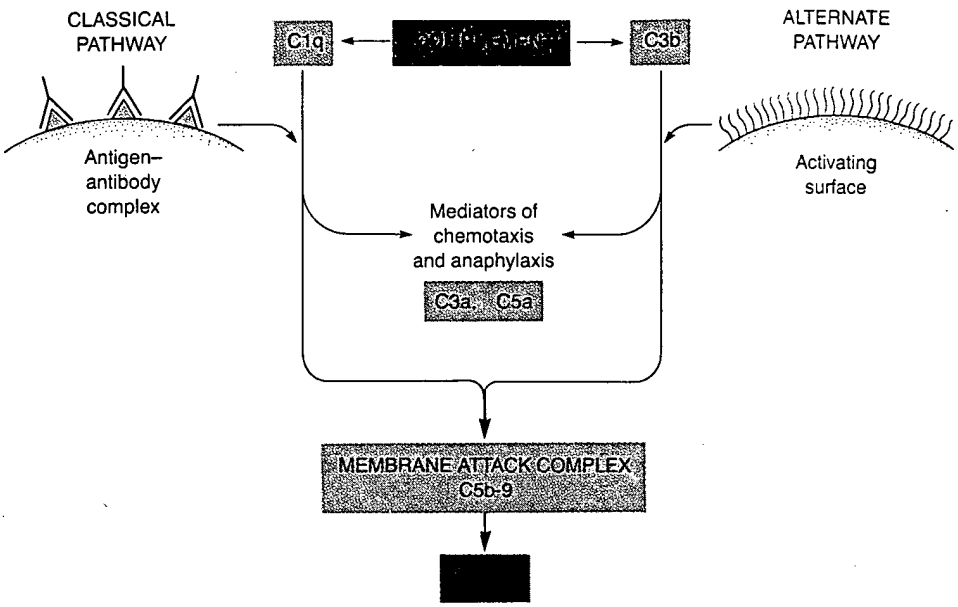


Figure 2-12. The complement system.

assembled. **Thus, whether the alternative or the classical complement pathway is activated, the end result is the same: formation of a membrane attack complex capable of inducing cell lysis and generation of the biologically active anaphylatoxins C3a and C5a.**

Anaphylatoxins

The anaphylatoxins C3a, C4a, and C5a are important products of complement activation via the classical pathway. Each of these molecules has been shown to have potent effects on smooth muscle and the vasculature, including enhancement of smooth muscle contraction and increasing vascular permeability (Fig. 2-13). Both C3a and C5a also induce mast cell and basophil degranulation, and the consequent release of histamine further potentiates the increase in vascular permeability. In addition to their effects on vascular smooth muscle, the anaphylatoxins stimulate contraction of bronchial smooth muscle and cause airway narrowing. This effect is produced in two ways. The first is dependent on arachidonic acid metabolism in the lung; the second is mediated by the release of mast cell products.

C5a is also a potent chemotactic factor for neutrophils, monocytes, eosinophils, and basophils and induces low levels of neutrophil degranulation and superoxide anion production. Additional effects of C5a stimulation of neutrophils include enhancement of both the phagocytic response and, in response to a second stimulus, degranulation and superoxide anion production. This enhancement effect is referred to as "cell priming." C3a and C5a also modulate certain immune responses. Whereas C3a inhibits T-lymphocyte proliferation, C5a promotes immune reactions. These immune-regulatory properties of C3a and C5a are discussed in greater detail in Chapter 4.

Table 2-1 Activators of the Complement System

CLASSICAL	ALTERNATIVE
Immune complexes (IgM, IgG)	Zymosan (yeast cell wall)
Aggregated antibody	Cobra venom factor (CVF)
Proteases	Endotoxin (lipopolysaccharides)
Urate crystals	Polysaccharides
Polyanions (polynucleotides)	X-ray contrast media
	Dialysis membranes
	Parasites, fungi, and viruses

Regulation of the Complement System

Activation of the complement system is regulated by three mechanisms. One mechanism involves the spontaneous decay of the individual enzymatically active complexes C4b2a, C3bBb, or cleavage products C3b and C4b. A second regulatory mechanism involves the proteolytic inactivation of specific components by inhibitors present in plasma, including factor I (an inhibitor of C3b and C4b) and serum carboxypeptidase N (SCPN). SCPN cleaves the carboxy-terminal arginine from the anaphylatoxins C4a,

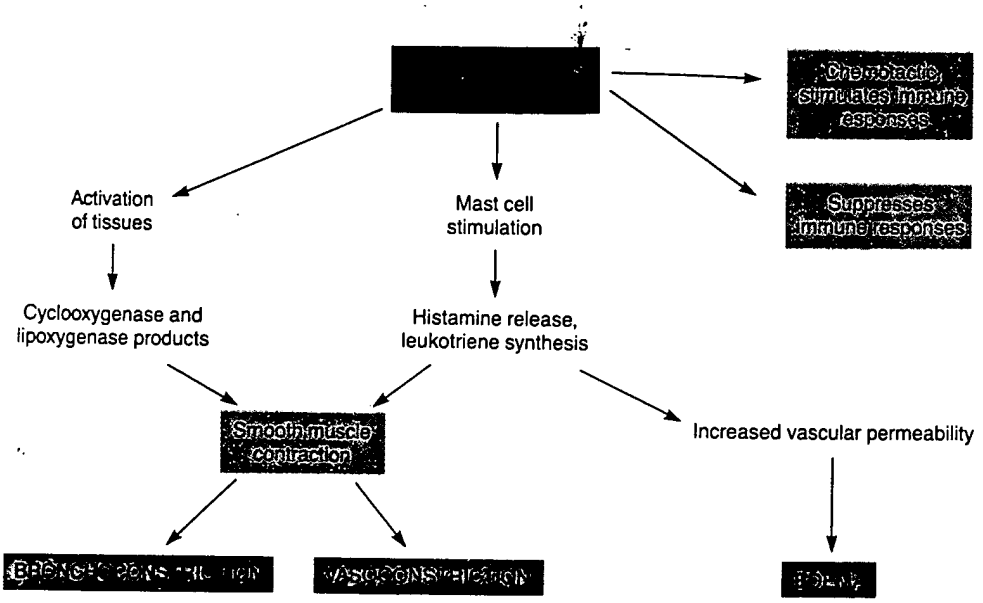


Figure 2-13. Biologic activity of the anaphylatoxins.

C3a, and C5a in a manner similar to that of bradykinin. Cleavage of the single amino acid markedly decreases the biologic activity of each of these molecules. A third mechanism of regulation of the complement system relates to the binding of active components by specific proteins in the plasma. The C1 esterase inhibitor (C1INA) regulates the activation of the classical pathway by binding C1r and C1s, forming an irreversibly inactive complex. Additional binding proteins present in plasma include factor H and C4b binding protein. These proteins form complexes with C3b and C4b, respectively, and enhance their susceptibility to proteolytic cleavage by factor I.

The complement system plays an important role in many forms of immunologic tissue injury (Chapter 4). In addition, it is an important host defense mechanism against bacterial infection. Bacterial activation of the complement system may occur either by direct activation of the alternative pathway or as an outcome of antibody binding to the surface of the bacterium and activation of the classical pathway. Once the complement system is activated, bacteriolysis may follow, either by means of the assembled membrane attack complex or by enhanced bacterial clearance following opsonization. Bacterial **opsonization** is the process by which a specific molecule (e.g., IgG or C3b) binds to the surface of the bacterium. The process enhances phagocytosis by enabling receptors on the phagocytic cell membrane (e.g., the Fc or the C3b receptor) to recognize and bind to the opsonized bacterium. Viruses, parasites, and transformed cells also activate the complement system by similar mechanisms, resulting in their inactivation or death.

The importance of an intact and appropriately regulated complement system as a component of host defense is exemplified in individuals who have deficiencies of either specific complement components or regulatory proteins. **Deficiencies of complement components may be either acquired or congenital.** The most common congenital defect is a C2 deficiency, which is inherited as an autosomal codominant trait with a gene frequency of approximately 1%. Acquired deficiencies of early complement components may occur in patients with certain autoimmune diseases, especially those associated with circulating immune complexes. These include certain forms of membranous glomerulonephritis and systemic lupus erythematosus. Patients with congenital deficiencies in the early components of the complement system have recurrent symptoms resembling those of systemic lupus erythematosus. Patients with deficiencies of the middle (C3, C5) and terminal (C6, C7, or C8) complement components are particularly susceptible to pyogenic bacterial and neisserial infections, respectively—a circumstance that indicates the importance of individual components of the complement system in host surveillance against bacterial infection. Congenital defects have been reported in regulatory proteins of the complement system, including deficiencies of C1INA and SCPN. Deficiency of C1INA is associated with the syndrome of hereditary angioedema. The syndrome is characterized by episodic, painless, nonpitting edema of soft tissues, particularly the subepithelial areas, gastrointestinal tract, and upper respiratory areas. It may become life threatening if the larynx is affected.

Phospholipid Metabolism and Arachidonic Acid Metabolites

Among the mediators generated by inflammatory cells and injured tissues, certain derivatives of phospholipids and fatty acids are important. Depending on the specific inflammatory cell and the nature of the stimulus, activated cells generate arachidonic acid by one of two pathways (Fig. 2-14). The first pathway involves stimulus-induced activation of phospholipase A_2, which enhances the hydrolysis of arachidonic acid from the glycerol backbone of membrane phospholipids. In particular, phosphatidylcholine is an important substrate of phospholipase A_2 and is thus the major source of arachidonic acid in inflammatory cells. The second mechanism by which arachidonic acid is generated is the metabolism of phosphatidylinositol by phospholipase C to diacylglycerol and inositol phosphates. Diacylglycerol lipase then cleaves arachidonic acid from diacylglycerol. Once generated, arachidonic acid, a polyunsaturated (20:4) fatty acid, is metabolized via two pathways: cyclo-oxygenation, with the subsequent production of prostaglandins and thromboxanes; and lipoxygenation, to form monohydroxyeicosatetranoic and dihydroxyeicosatetranoic acids (HETEs and diHETEs) and leukotrienes.

Specific cyclo-oxygenase enzymes in inflammatory cells generate endoperoxide derivatives of arachidonic acid, including prostaglandin G_2 (PGG_2) and prostaglandin H_2 (PGH_2). These endoperoxides are unstable and, depending on the specific inflammatory cell or tissue, are metabolized to more stable prostaglandins, including PGI_2 (also known as prostacyclin), $PGF_{2\alpha}$, PGE_2, PGD_2 and thromboxane A_2 (TxA_2). The primary cyclo-oxygenase metabolite in platelets is thromboxane A_2; endothelial cells secrete principally PGI_2. Macrophages, depending on their state of activation, produce any or all of these derivative products.

PGI_2 and PGE_2, owing to their vasodilatory effects, enhance vascular permeability at sites of inflammation; thromboxane A_2 is a potent vasoconstrictor and plays an important role in the mediation of the "second wave" of platelet aggregation. PGI_2 and PGE_2 bind to specific receptors on inflammatory cells, thereby activating adenylate cyclase and increasing intracellular cyclic adenosine monophosphate (cAMP) levels, thereby inhibiting their functional responses to other inflammatory stimuli.

A second pathway by which arachidonic acid is metabolized in inflammatory cells and tissues is lipoxygenation and the formation of hydroperoxyeicosatetranoic acid compounds (HPETEs). Hydroperoxy- compounds may be metabolized to hydroxyeicosatetranoic acids (HETEs) or to leukotriene A_4, which contains three conjugated double bonds and serves as a precursor for other leukotriene molecules. In the neutrophil and in certain macrophage populations, leukotriene A_4 is metabolized to leukotriene B_4, a compound with potent chemotactic activity for neutrophils, monocytes, and macrophages. In other cell types, especially mast cells, basophils, and macrophages, the addition of glutathione to leukotriene A_4 results in the formation of leukotriene C_4. Leukotrienes D_4 and E_4 are formed following sequential removal of the amino acids glycine and glutamine, respectively. LTC_4, LTD_4, and LTE_4 are collectively known as **slow-reacting substances of anaphylaxis (SRS-As).** They stimulate the contraction of smooth muscle and enhance vascular permeability. The generation of leukotriene B_4 at sites of tissue injury plays an important role in the recruitment of polymorphonuclear leukocytes, while the production of leukotrienes C_4, D_4, and E_4 is responsible for the development of much of the clinical symptomatology associated with allergic-type reactions.

The importance of arachidonic acid metabolites in mediating many of the effects of the inflammatory response is demonstrated by the ability of inhibitors of the involved enzymes to attenuate both the pathologic changes and clinical symptomatology (Table 2-2). Corticosteroids are widely used to inhibit the tissue destruction associated with many inflammatory diseases, including allergic responses, rheumatoid arthritis, and systemic lupus erythematosus. Corticosteroids induce the synthesis of an inhibitor

Table 2-2 Biologic Activity of Arachidonic Acid Metabolites

METABOLITE	BIOLOGIC ACTIVITY
PGE_2, PGD_2	Induce vasodilation, bronchodilation Inhibit inflammatory cell function
PGI_2	Induces vasodilation, bronchodilation Inhibits inflammatory cell function
$PGF_{2\alpha}$	Induces vasodilation, bronchoconstriction
TxA_2	Induces vasoconstriction, bronchoconstriction Enhances inflammatory cell functions (esp. platelets)
LTB_4	Chemotactic for phagocytic cells Stimulates phagocytic cell adherence Enhances microvascular permeability
LTC_4, LTD_4, LTE_4	Induce smooth-muscle contraction Constrict pulmonary airways Increase microvascular permeability

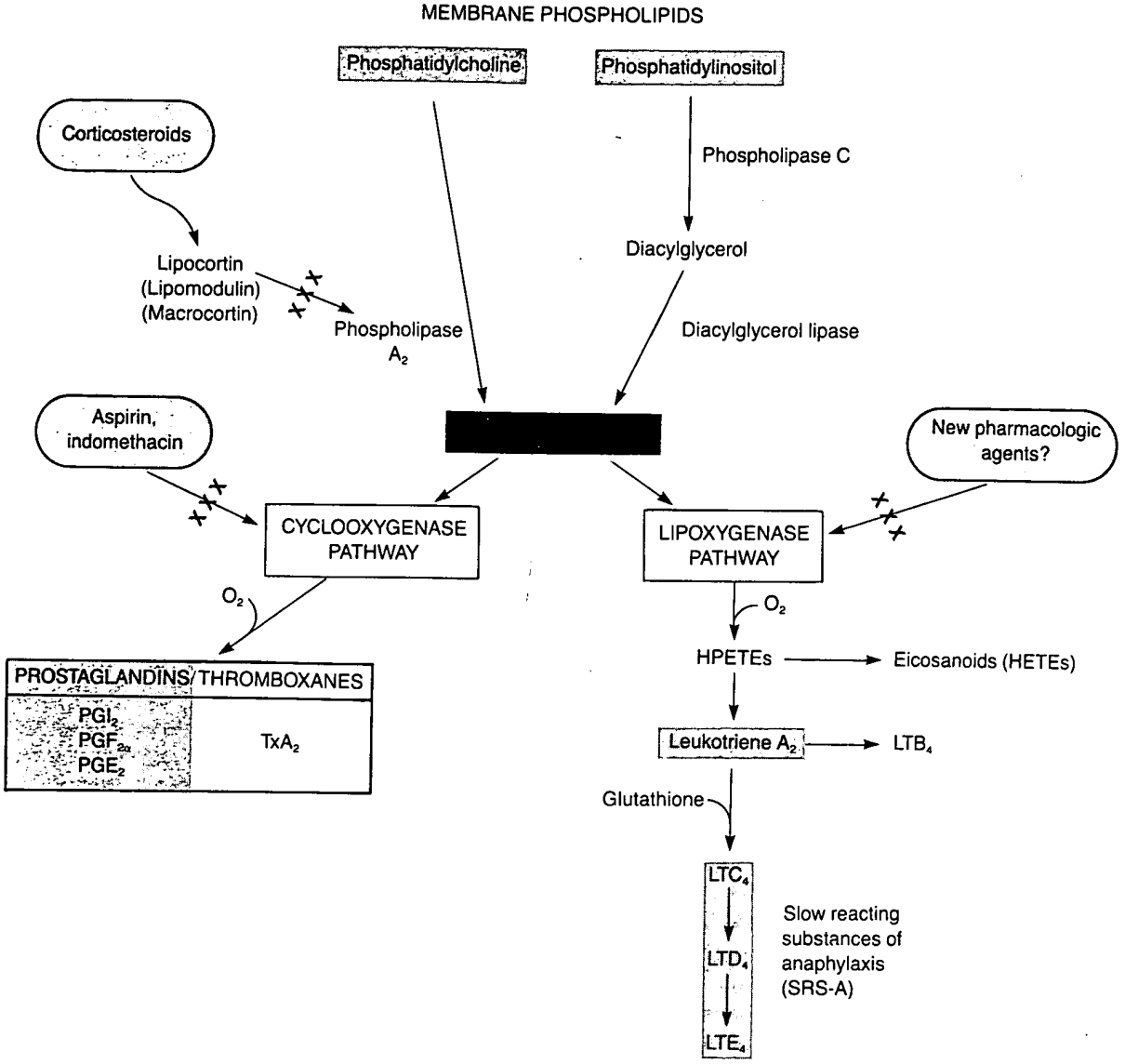


Figure 2-14. Arachidonic acid metabolism.

of phospholipase A_2 and block the release of arachidonic acid in inflammatory cells. Originally described as two proteins, lipomodulin and macrocortin, this regulatory inhibitor is now known to be a single protein, referred to as **lipocortin.**

A second class of anti-inflammatory agents that is widely used in the treatment of inflammatory diseases comprises the nonsteroidal anti-inflammatory drugs. These compounds—including aspirin, indomethacin, ibuprofen, and piroxicam—inhibit cyclooxygenase, and thus the synthesis of prostaglandins and thromboxanes. A third class of compounds that block specific lipoxygenase activities in inflammatory cells is currently being developed.

Cellular Recruitment

The second phase of the acute inflammatory response involves the accumulation of leukocytes—especially polymorphonuclear leukocytes (PMNs)—at sites of tissue injury (Fig. 2-15). During the first 24 hours (and sometimes even in the first few hours) after initiation of injury, many polymorphonuclear leukocytes accumulate. The sequence of events leading to this accumulation at inflammatory sites is initiated by locally generated soluble chemical mediators. These mediators, collectively referred to as **chemotactic factors,** are generated in high concentra-

tions at sites of tissue injury, with a progressively decreasing gradient away from the injured tissue.

The physiologic responses of circulating leukocytes exposed to chemotactic factors include margination of the cells along the vascular wall, adherence of the leukocytes to the endothelium or vascular basement membrane, emigration through the vascular wall, and unidirectional migration toward increasing concentrations of the chemotactic agent (**chemotaxis**). The most important chemotactic factors for polymorphonuclear leukocytes are C5a derived from complement, low-molecular-weight N-formylated peptides (such as N-formyl-methionyl-leucyl-phenylalanine) derived from bacteria and mitochondria, and specific products of lipid metabolism, including leukotriene B_4.

Chemotactic factors for other cell types, including monocytes, lymphocytes, basophils, and eosinophils, are also produced at sites of tissue injury. Low-molecular-weight secretory products of lymphocytes (referred to as lymphokines) are chemotactic, as are secretions of monocytes and tissue macrophages (monokines). Proteases from neutrophils and macrophages also cleave C5 to generate C5a or C5a-related peptides.

Inflammatory Cell Activation

The polymorphonuclear leukocyte, mast cell, mononuclear phagocytic cells and platelet are important cellular components of the inflammatory reaction. Once stimulated, these cells release inflammatory mediators that cause tissue injury.

The polymorphonuclear leukocyte is activated in response to phagocytic stimuli or by binding of chemotactic mediators or antibody–antigen complex to specific receptors on its cell membrane. Neutrophil receptors react with the Fc portion of IgG and IgM molecules; with complement system components C5a, C3b, and C3bi; with arachidonic acid metabolites (e.g., leukotriene B_4); and with formylated low-molecular-weight chemotactic peptides.

Mast cells, platelets, and mononuclear phagocytic cells are also activated in a receptor-specific manner. **The process by which diverse stimuli lead to the functional responses of inflammatory cells (e.g., degranulation or aggregation) is referred to as "stimulus–response coupling."** Common pathways associated with inflammatory cell activation are stimulus-induced increases in phospholipid metabolism of cell membranes, raised intracellular calcium levels, and augmented protein kinase activity within the cell (Fig. 2-16).

The binding of a chemotactic factor to a specific receptor on the cell membrane results in the formation of a ligand–receptor complex (Fig. 2-17). A guanine-nucleotide regulatory protein couples the ligand–receptor complex to a specific phosphodiesterase in the inflammatory cell membrane, a process that activates the esterase. In the neutrophil this phosphodiesterase activity is phospholipase C. Stimulus-induced activation of phospholipase C enhances phosphoinositide turnover and the formation of two potent metabolites, diacylglycerol and inositol trisphosphate. Inositol trisphosphate releases calcium from intracellular stores. The release of intracellular calcium in conjunction with an influx of calcium ions from the extracellular environment contributes to an increase in free intracellular calcium—a critical event for the activation of most inflammatory cells. The increase in free intracellular calcium may have many effects, including the potentiation of phospholipase A_2 activity and the activation of multiple protein kinases that phosphorylate a variety of proteins. In addition, calmodulin (a high-affinity calcium-binding protein of nonmuscle cells) is important in the activation of inflammatory cells. The mobilization of intracellular calcium is also closely linked to the activation of cytoskeletal elements. Assembly of the microtubular system and activation of actin–myosin complexes are crucial for the secretion of cytoplasmic granules and for chemotaxis of neutrophils and other inflammatory cells.

A second product of phospholipase C activity, diacylglycerol, mediates additional responses within the cell. Diacylglycerol activates protein kinase C, a cytosolic enzyme that associates with the cell membrane following cell stimulation. Protein kinase C phosphorylates several substrate proteins within the cell, including cytoskeletal elements, and alters their functional properties.

As described above, phospholipase A_2 is also activated by stimulation of inflammatory cells. This calcium-activated phospholipase releases arachidonic acid from membrane phospholipids; its subsequent metabolism to biologically active compounds is important in both inflammatory cell activation and the development of the inflammatory response.

Phospholipase A_2 activity is regulated by lipocortin (see Fig. 2-14). The dephosphorylated form of lipocortin inhibits phospholipase A_2 activity and the release of arachidonic acid. Thus, the phosphorylation of lipocortin during inflammatory cell activation frees phospholipase A_2 activity from inhibition, resulting in an increased release of arachidonic acid. The induction of elevated levels of dephosphorylated lipocortin within inflammatory cells has been postulated

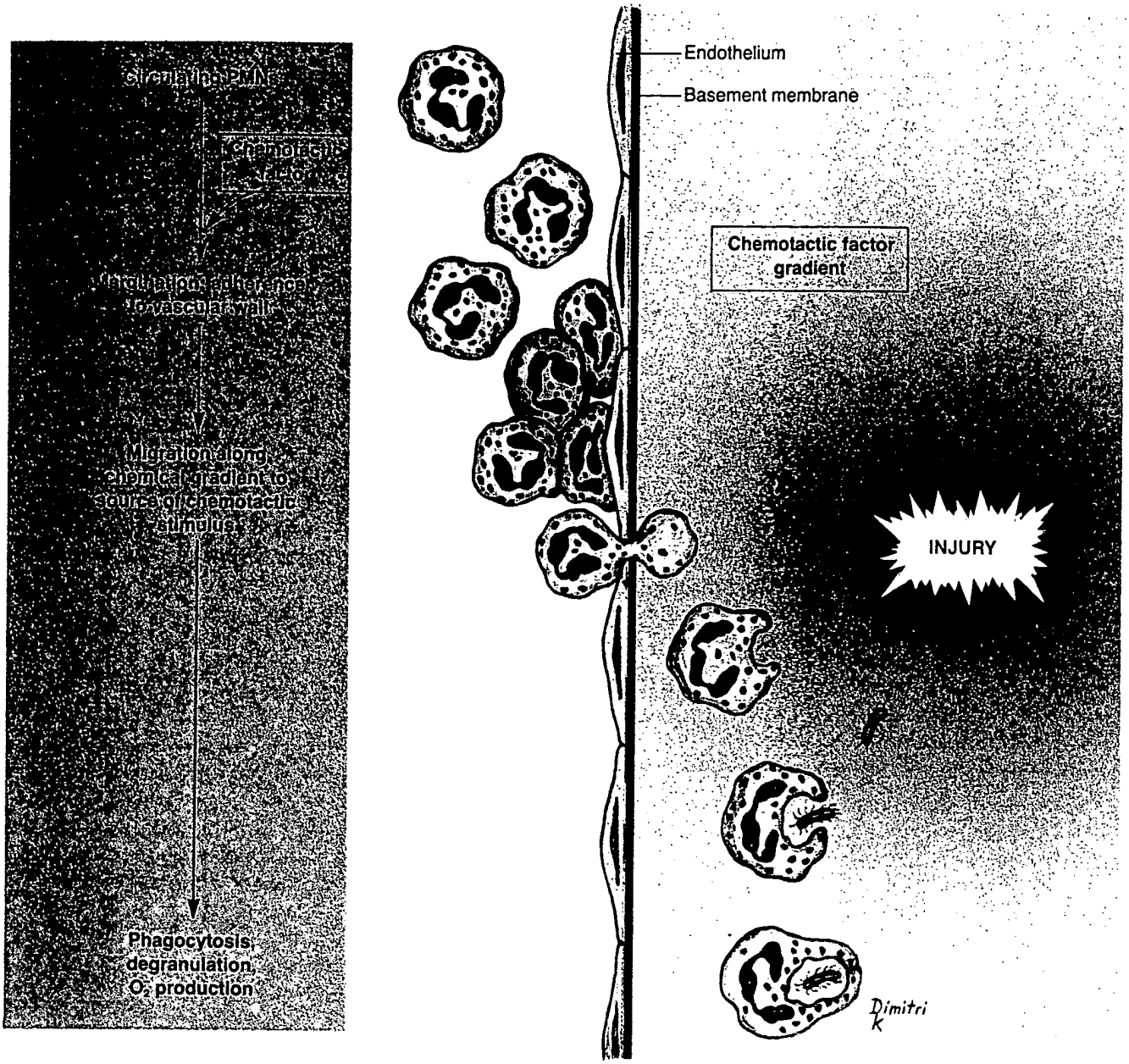


Figure 2-15. Leukocyte exudation and phagocytosis.

as a mechanism for the anti-inflammatory effects of corticosteroids.

The activation of methyltransferases following inflammatory cell stimulation also affects the phospholipid components of inflammatory cell membranes. Two methyltransferases that catalyze the methylation of phosphatidylethanolamine to phosphatidylcholine have been identified in the mast cell and basophil. The generation of phosphatidylcholine via transmethylation reactions is thought to be important for antigen-induced degranulation of mast cells and basophils.

An understanding of inflammatory cell stimulation will provide the basis for new strategies for therapeutic modulation of inflammation in human disease. For instance, specific lipoxygenase or phospholipase inhibitors could be developed to inhibit the early activation processes of inflammatory cells, thus suppressing the tissue injury associated with certain diseases.

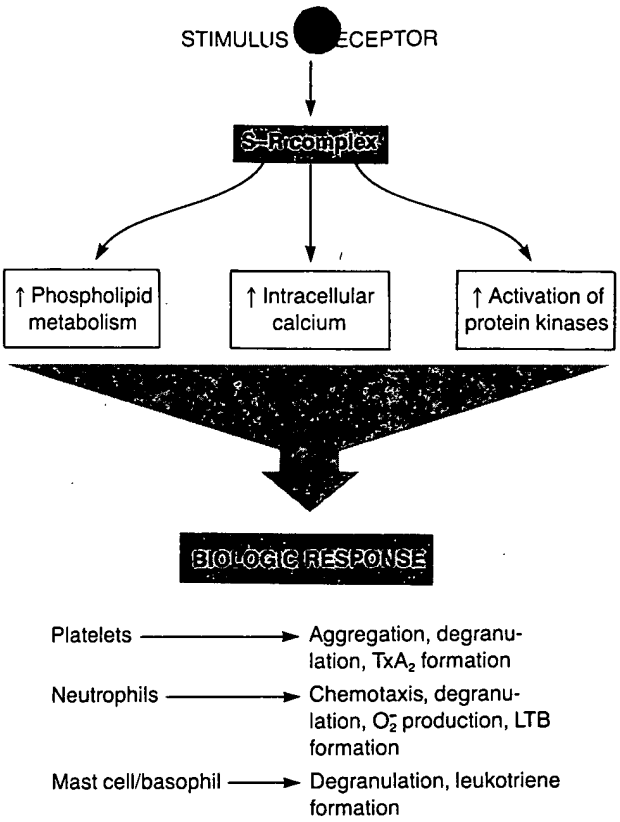


Figure 2-16. Mechanisms of inflammatory cell activation.

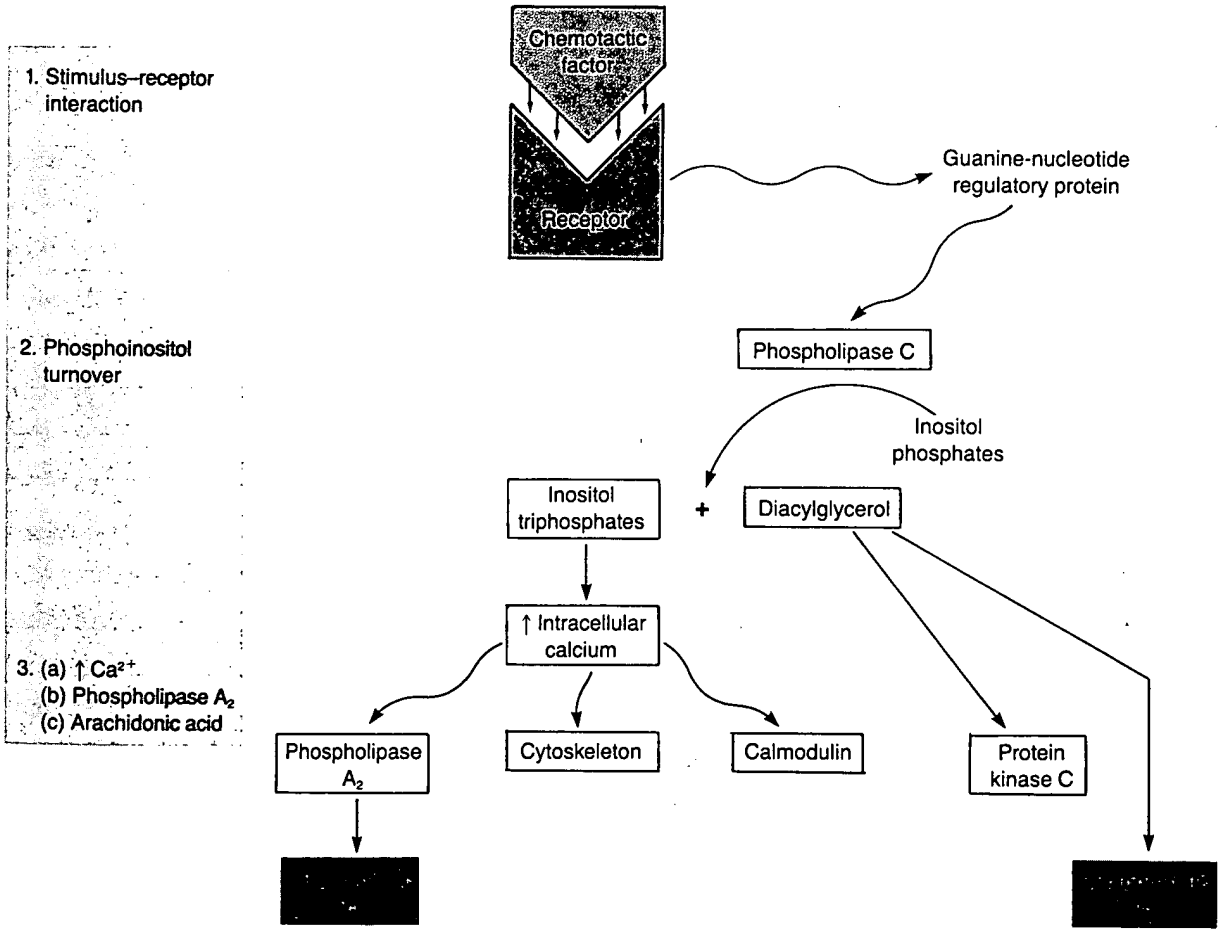


Figure 2-17. Initial events in polymorphonuclear leukocyte activation.

Modulation of Inflammatory Cell Function

The pharmacologic modulation of inflammatory cell activation is exemplified by the inhibitory effects of E-series prostaglandins and PGI_2 on the functional responses of mast cells, neutrophils, macrophages, and platelets. Prostaglandins of the E series and PGI_2 inhibit inflammatory cell function by activating adenylate cyclase and increasing intracellular cAMP levels (Fig. 2-18). Beta-adrenergic agonists and cholera toxin also stimulate adenylate cyclase activity and inhibit inflammatory cell function. Furthermore, treatment of inflammatory cells with an inhibitor of cyclic nucleotide phosphodiesterase, such as theophylline, increases levels of cAMP within the cells and enhances inhibition by adenylate cyclase agonists of cell functional responses.

Adenylate cyclase agonists and phosphodiesterase inhibitors also affect target tissues of inflammatory mediators. For instance, an increased cAMP level in bronchiolar smooth muscle causes relaxation and blocks the effects of mediators that promote bronchoconstriction. This is the basis for using phosphodiesterase inhibitors in the pharmacologic modulation of mast cell degranulation and the arrest of clinical symptoms associated with certain allergic reactions. In contrast, mediators that activate guanylate cyclase and increase intracellular cyclic guanosine monophosphate (GMP) levels, such as thromboxane and acetylcholine, enhance inflammatory cell function. **In summary, the ability of both the inflammatory cell and the target tissue to respond to a specific inflammatory mediator is modulated by intracellular cyclic nucleotides and is subject to pharmacologic modulation of the inflammatory response.**

Prostaglandin I_2 (PGI_2), a secretory product of endothelial cells, inhibits activation of neutrophils and platelets, maintains vascular integrity, and modulates recruitment of neutrophils to sites of inflammation (Fig. 2-19). Under normal conditions PGI_2 increases intracellular cAMP levels and suppresses platelet and polymorphonuclear leukocyte activation within the vascular compartment. Any pathologic condition that decreases the endothelial production of PGI_2, de-

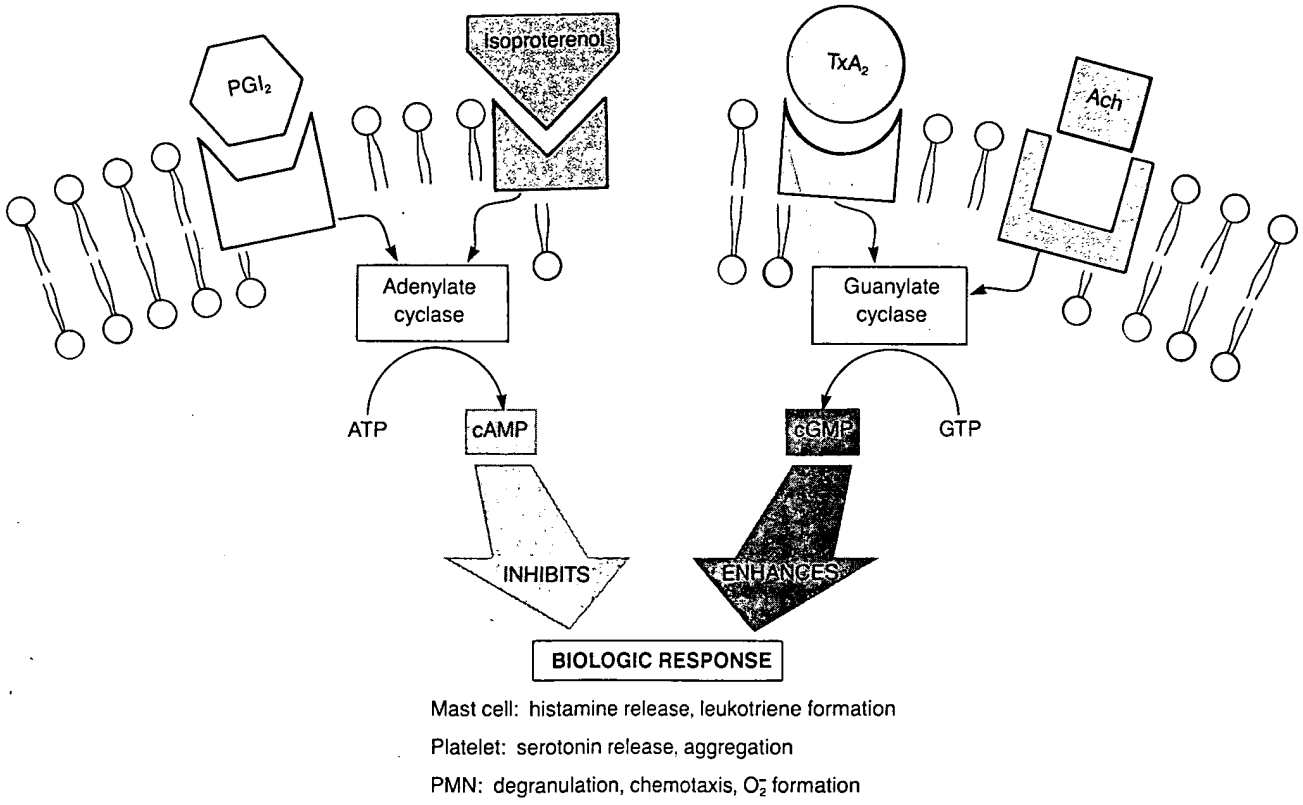


Figure 2-18. The biologic response of inflammatory cells is modulated by activating and inhibitory cyclic nucleotides.

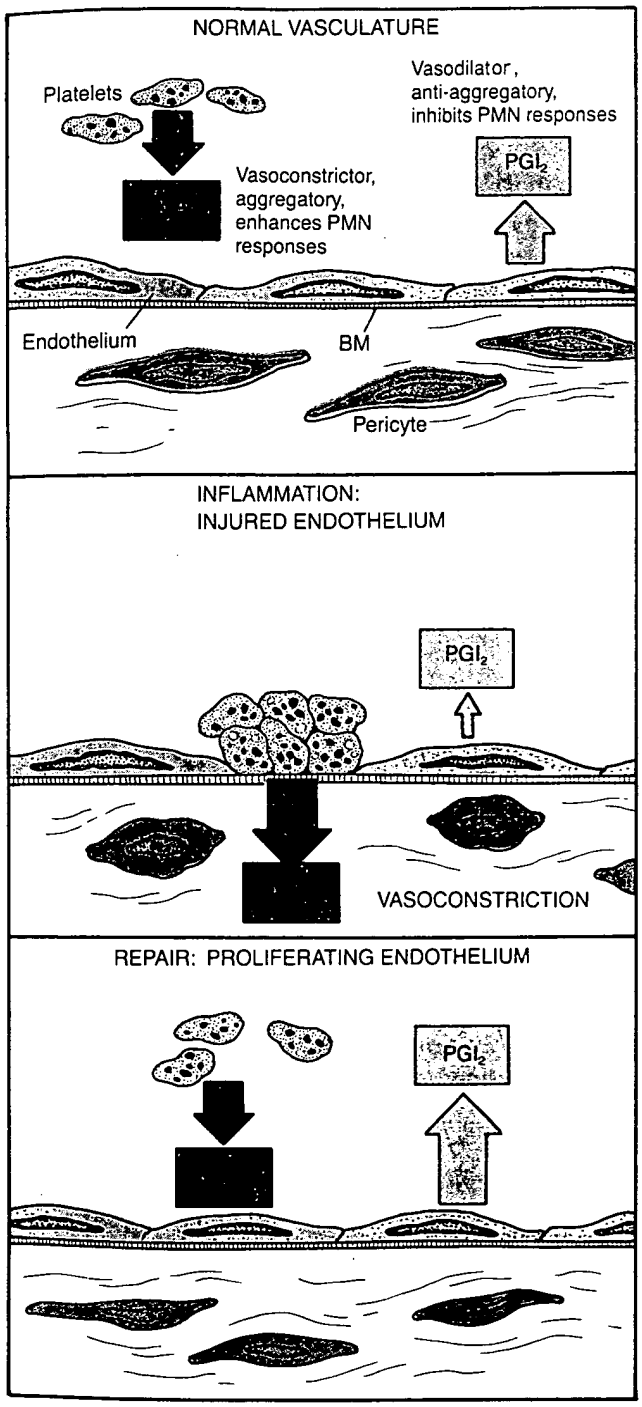


Figure 2-19. Regulation of platelet and endothelial cell interactions by thromboxane A_2 and prostaglandin I_2. During inflammation the normal balance is shifted to vasoconstriction, platelet aggregation and polymorphonuclear leukocyte responses. During repair the prostaglandin effects predominate.

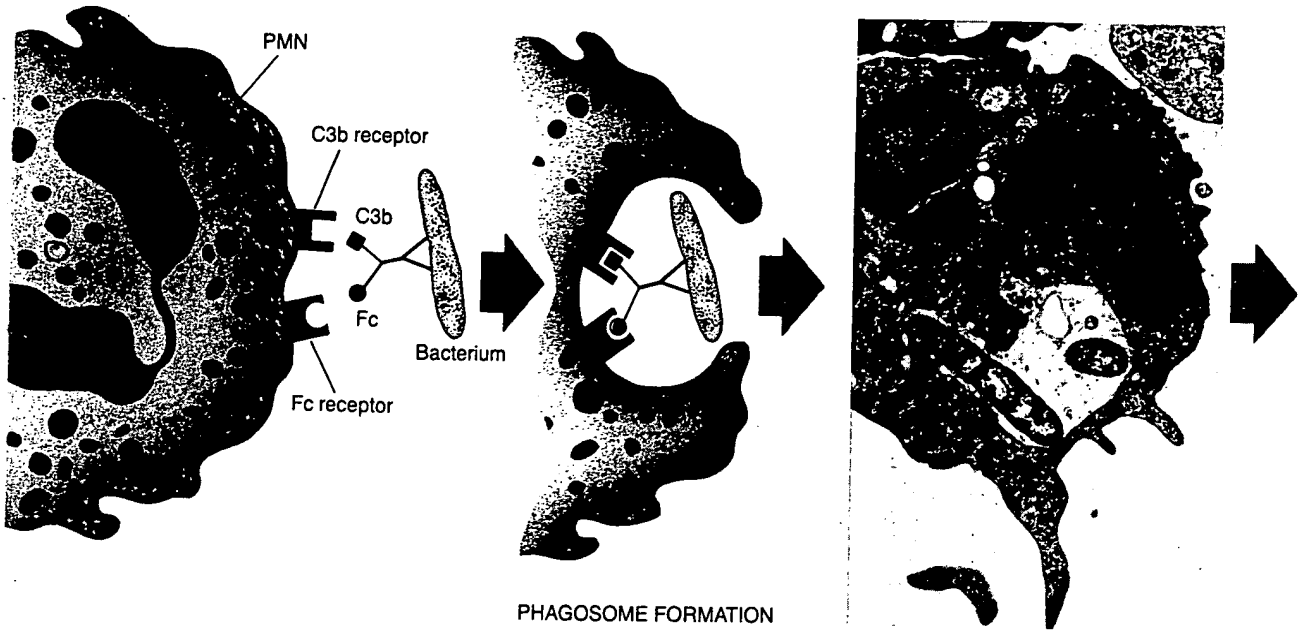


Figure 2-20. Mechanisms of polymorphonuclear leukocyte bacterial phagocytosis and cell killing.

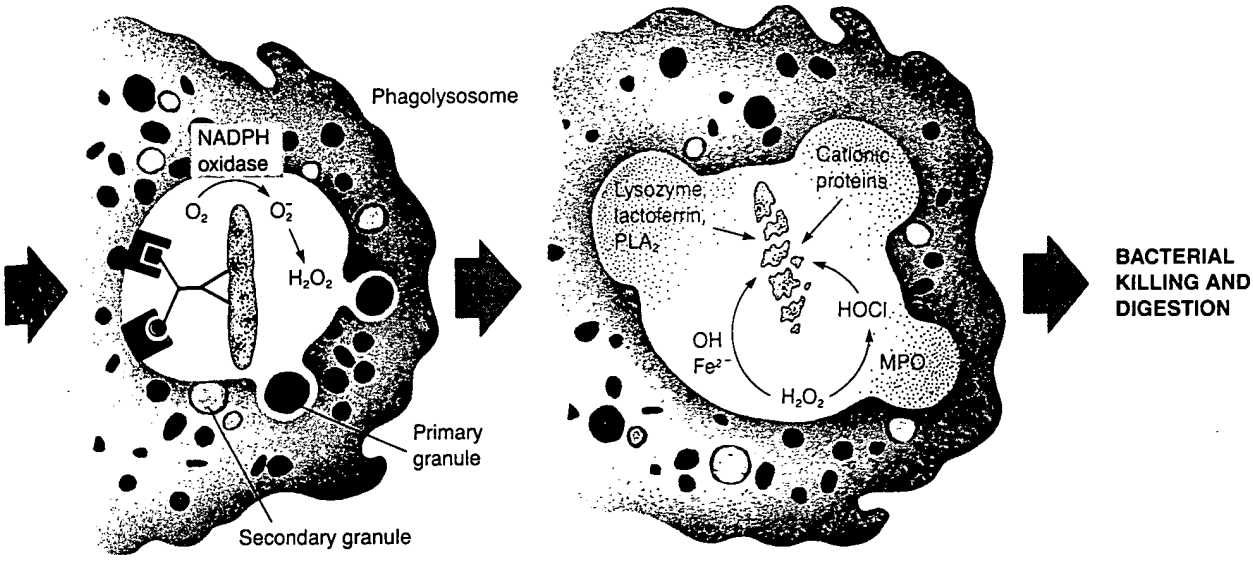
creases the sensitivity of platelets and polymorphonuclear leukocytes to PGI_2, or presents an overwhelming inflammatory stimulus (e.g., high levels of C5a or thrombin) promotes functional responses of both cell types. Alterations in the homeostatic balance between platelet stimulation and suppression by PGI_2 have been postulated as important in the development of vascular thrombi and the pathogenesis of atherosclerosis and its complications. Similar alterations in the regulation of polymorphonuclear leukocyte activation by PGI_2 probably function in the initiation of acute inflammatory reactions.

Mechanisms of Injury Produced by Polymorphonuclear Leukocytes

The process of engulfment and internalization of foreign agents or injured cell material is termed phagocytosis, and cells that possess this function are referred to as phagocytic cells. Among these cells are polymorphonuclear leukocytes, monocytes, and tissue macrophages (including Kupffer's cells).

The process of phagocytosis of a bacterium or foreign material may be enhanced by the opsonization of that particle by antibody or the fixation of C3b on its surface (Fig. 2-20). As described above, phagocytic cells possess specific receptors for C3b, C3bi, and the Fc fragment of immunoglobulin molecules. The engulfment of a foreign agent by the cell membrane results in the formation of a phagosome, which then fuses with a lysosome to form a phagolysosome. The release of lysosomal contents into the phagolysosome exposes the engulfed particle to the degradative properties of lysosomal hydrolases, which are activated by acidification within the phagolysosome.

Three distinct granules in the cytoplasm of polymorphonuclear leukocytes are designated the primary, secondary, and tertiary granules, respectively (Fig. 2-21). Each granule displays a unique spectrum of enzymes. **Primary granules** contain potent acid hydrolases capable of digesting mucopolysaccharides. These granules also contain elastase and cathepsin G, which are serine proteases capable of digesting structural proteins of tissues, including elastin and collagen. Also present in these granules are cationic proteins that enhance the adherence of neutrophils to targets and initiate cytotoxic injury of certain cell types. Both these functions of the cationic proteins play an important role in killing bacteria. Other enzymes in the primary granule with known bactericidal activity are lysozyme and phospholipase A_2, which degrade bacterial cell walls and membranes, respectively. As noted previously, phospholipase A_2 also promotes vascular permeability in skin sites and plays an important role in the degradation of eukaryotic cell membranes at sites of tissue injury.

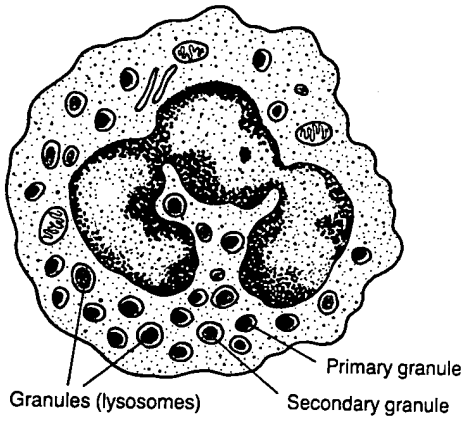


DEGRANULATION AND NADPH OXIDASE ACTIVATION

Myeloperoxidase, an enzyme also present in these granules, enhances cytotoxicity by metabolizing hydrogen peroxide in the presence of halide ions (e.g., Cl^- or I^-) to form hypohalous acid (see below). **Secondary granules** also contain phospholipase A_2, lysozyme, the cationic protein lactoferrin, a vitamin B_{12}-binding protein, and a collagenase against type IV collagen. **Tertiary granules,** also referred to as C particles, contain acid hydrolases and gelatinase, the latter an enzyme that digests basement membrane and denatured collagen. Tertiary granules are released at the leading front of neutrophils during chemotaxis and are thought to be the source of enzymes that promote the migration of cells through basement membranes and tissues. Similar granules are present in monocytes and macrophages. Secondary and perhaps tertiary granules also contain chemotactic receptors that can be added to the neutrophil cell membrane during fusion of lysosomal granules. Monocytes possess myeloperoxidase, but tissue macrophages do not. Both monocytes and macrophages contain varying amounts of acid hydrolase activity, collagenase, and gelatinase. However, there is a paucity of elastase and cathepsin G in both cell types. An additional neutral protease, plasminogen activator, is also secreted by both neutrophils and mononuclear phagocytic cells. Plasmin, the product of the cleavage of plasminogen by plasminogen activator, attacks several substrates, including fibrin, complement, and fibronectin. Therefore, both neutrophils and mononuclear phagocytic cells have the ability to degrade fibrin at sites of tissue injury and activate the complement system.

The biologic activities of proteases, such as elastase and cathepsin G, are regulated by inhibitors in plasma and tissue fluids. α_1-antiprotease is synthesized by hepatocytes and is the primary inhibitor of neutrophil elastase. The importance of this protein's inhibitory activity is evident in patients with a genetic deficiency of α_1-antiprotease: such patients develop pulmonary emphysema, presumably as a result of the lack of elastase inhibitory activity. A second antiprotease in plasma is α_2-macroglobulin, a protein that also inhibits elastase and cathepsin G activities. Additionally, both α_1-antiprotease and α_2-macroglobulin inhibit plasmin, trypsin, and chymotrypsin activities. Thus, circulating serum inhibitors not only block protease activity derived from phagocytic cells, but also inhibit protease activity generated within the plasma and tissues.

In addition to releasing granular enzymes into the phagolysosome, phagocytosis activates an NADPH oxidase in the cell membrane (see Fig. 2-20). The activation of this enzyme is associated with an increase in oxygen consumption and activation of the hexose-monophosphate shunt. Together these cell responses are referred to as the **"respiratory burst."** The activation of the enzyme is enhanced by prior



CHARACTERISTICS AND FUNCTIONS

- Acute inflammation
- Bacterial and foreign body phagocytosis

PMN INFLAMMATORY MEDIATORS

- Reactive oxygen metabolites
- Lysosomal granule contents
  - **–Primary granules**
    - Phospholipase A_2
    - Myeloperoxidase
    - Lysozyme
    - Cationic proteins
    - Acid hydrolases
    - Elastase
    - Cathepsins
  - **–Secondary granules**
    - Phospholipase A_2
    - Lysozyme
    - Alkaline phosphatase
    - Collagenase
    - Lactoferrin
    - Vitamin B_{12} binding proteins
  - **–Tertiary or C particles**
    - Gelatinase
    - Cathepsins

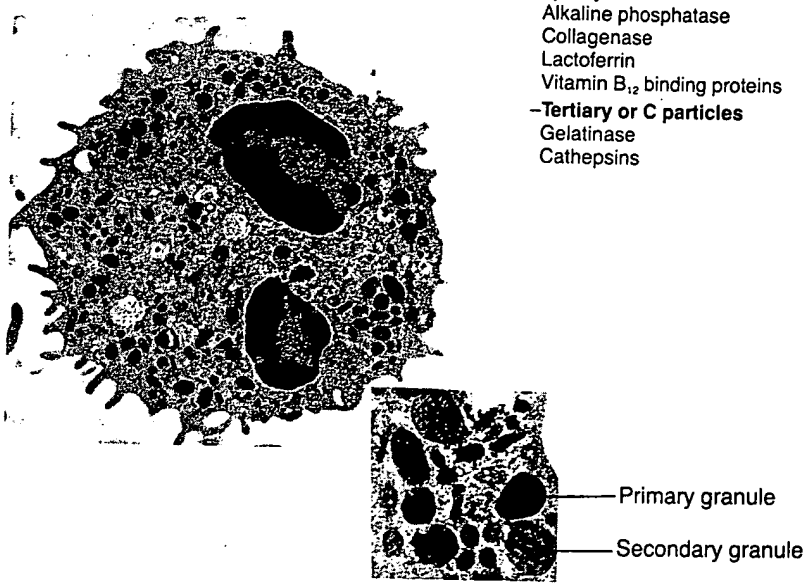


Figure 2-21. Polymorphonuclear leukocyte: morphology and function.

exposure of the cells to small amounts of a chemotactic stimulus or bacteria-derived lipopolysaccharide. NADPH oxidase reduces molecular oxygen to the superoxide anion, O_2^-. Almost all the oxygen consumed following initiation of the respiratory burst can be accounted for by the generation of O_2^-.

Superoxide anion is reduced to hydrogen peroxide via a dismutation reaction at the cell surface and within phagolysosomes. As discussed above, hydrogen peroxide, which is also a product of the respiratory burst, can react with myeloperoxidase in the presence of a halide to form hypohalous acid (Table 2-3). The most prominent halogen present in biologic systems is chlorine, and hypochlorous acid is produced following neutrophil stimulation. This acid is a more potent oxidant and bactericidal agent than hydrogen peroxide. Hypochlorous acid, in addition to granule proteases, activates neutrophil-derived collagenase and gelatinase, both of which are secreted as latent enzymes. The reaction of hypochlorous acid with molecules containing free amino groups produces monochloramine and dichloramine compounds. These stable compounds oxidize sulfhydryl residues to dialdehydes and thioethers to sulfoxides. As noted in Chapter 1, further reduction of H_2O_2 occurs via a Haber-Weiss reaction that forms the highly reactive hydroxyl radical (·OH). At physiologic pH this reaction occurs slowly. However, reduction of hydrogen peroxide is facilitated by reduced transition metals, such as ferrous iron. In the presence of Fe^{2+} a Fenton-type reaction converts H_2O_2 to ·OH, a radical with potent bactericidal activity. Further reduction of ·OH leads to the formation of H_2O. The mechanisms by which reactive oxygen

Table 2-3 Reactions Involving Reactive Oxygen Metabolites Produced by Phagocytic Cells

Reduction of Molecular Oxygen	
$O_2 + e^- \rightarrow O_2^-$	Superoxide anion
Dismutation of O_2^-	
$O_2^- + O_2^- + 2H^+ \rightarrow O_2 + H_2O_2$	Hydrogen peroxide
Haber-Weiss Reaction	
$H_2O_2 + O_2^- \rightarrow OH^- + \cdot OH$	Hydroxyl radical
Fenton Reaction (iron-catalyzed)	
$H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH^- + \cdot OH$	Hydroxyl radical
Myeloperoxidase Reaction	
$H_2O_2 + Cl^- + H^+ \rightleftharpoons H_2O + HOCl$	Hypochlorous acid

metabolites may initiate cell and tissue injury include initiation of lipid peroxidation, DNA scission and cross linking, sulfhydryl group oxidation in proteins, and depolymerization of glycosaminoglycans (e.g., hyaluronic acid).

Reactive oxygen metabolites and lysosomal enzymes are synergistic in producing tissue injury. Proteins and glycosaminoglycans exposed to oxidants are rendered more susceptible to degradation by proteases and acid hydrolases, respectively. For example, oxidants in cigarette smoke and from activated phagocytic cells react with a methionine residue on α-1-antiprotease to render it inactive. This inactivation of α-1-antiprotease enhances elastase activity at sites of tissue injury.

Monocytes, macrophages, and eosinophils also may produce superoxide anion and hydrogen peroxide, depending on their state of activation and the stimulus to which they are exposed. The production of reactive oxygen metabolites by these cells has been implicated in their bactericidal and fungicidal activity, as well as in their ability to kill certain parasites.

The importance of oxygen-dependent mechanisms in phagocytic cell function is exemplified in patients who suffer from a defect in NADPH oxidase. Chronic granulomatous disease of childhood is characterized by an inherited defect in NADPH oxidase or in its expression, and by a failure to produce superoxide anion and H_2O_2 during phagocytosis. Patients with this disorder are susceptible to recurrent infections, especially by gram-positive cocci (Tables 2-4, 2-5).

Depending on the nature of the tissue injury and the size of the phagocytized particle, varying amounts of lysosomal enzymes are released into the extracellular milieu. Prolonged activation of NADPH oxidase may result in the release of hydrogen peroxide and superoxide anion into the adjacent tissues. Under these conditions the activation of phagocytic cells is harmful to the host, since it leads to tissue damage. Such mechanisms of tissue injury play an important role in the pathogenesis of several diseases, including pulmonary emphysema, rheumatoid arthritis and other immune complex diseases, and adult respiratory distress syndrome.

Cell Adherence and Tissue Injury

The adherence of inflammatory cells to the endothelium or vascular basement membrane is critical for recruitment of these circulating cells to sites of tissue injury. Under normal conditions inflammatory cell membranes and vascular basement membrane possess mutually repulsive negative charges. The number of anionic sites (and hence, negative charges) on the surface of endothelial cells and basement membrane decreases after injury, resulting in a concomitant decrease in the repulsion between the circulating inflammatory cells and the vascular wall. Other com-

Table 2-4 Congenital Defects in Phagocytic Cell Function

Defect	Affected Group	Clinical Effect
Defect in NADPH oxidase: deficient O_2^-, H_2O_2 production	Those with chronic granulomatous disease of childhood (CGD). CGD has several forms; it is most commonly inherited as x-linked recessive	Increased risk of infection with pyogenic bacteria and fungi
Myeloperoxidase deficiency	Heterogenous group of patients	Increased risk of *Candida* infections
Defect in adherence proteins: MO-1, GP 150,95	Heterogenous group of patients	Increased risk of recurrent bacterial infections
Lysosomal granule defect	Those with Chédiak-Higashi syndrome, a rare disorder with large cytoplasmic inclusions	Defective bacterial killing and cell locomotion

Table 2-5 Causes of Acquired Defects in Phagocytic Cell Locomotion

Overwhelming infections
Severe trauma or burn
→Diabetes mellitus
→Chronic debilitating disease

ponents of plasma, vascular wall, and phagocytic cell membranes participate in modulating cell adherence reactions. Fibronectin, a glycoprotein present in plasma, basement membranes, and, to a lesser degree, phagocytic cell membranes, is important in modulating the attachment of phagocytic cells to vascular walls. Following injury to the vascular wall or tissues, increased amounts of fibronectin are deposited at the injury site. Fibronectin also is a potent opsonin that enhances phagocytosis of bacteria and phagocytic cell adherence.

Membrane glycoproteins that promote adherence are among the factors that enhance phagocytic cell attachment to vascular walls and phagocytic particles. The adherence glycoproteins of human leukocytes are a family of molecules consisting of alpha and beta subunits. The three best-studied molecules are designated MO-1, LFA-1 (leukocyte function antigen-1), and p150,95. In each of these molecules the beta chain is conserved; the specificity of each molecule for adherence properties is conferred by the alpha chain. Activation of phagocytic cells by chemotactic stimuli increases the expression of these adherence molecules on the cell surface. The importance of these glycoproteins in the adherence responses is demonstrated by two observations: When phagocytic cells are treated with antibodies to block expression of the surface adherence glycoproteins MO-1 and p150,95, their adherence to endothelial cells and foreign surfaces following chemotactic factor stimulation is inhibited. In addition, a group of individuals has been identified as possessing deficiencies of specific adherence glycoproteins. Persons deficient in MO-1 or GP 150,95 are susceptible to recurrent bacterial infection. Thus, the expression of these glycoproteins on phagocytic cell surfaces plays an important role in the localization of cells within the vasculature at sites of tissue injury and in the host defense against bacterial infection.

The adherence of phagocytic cells to their targets is associated with an increased cytotoxicity and degradation of substrates. The ability of a phagocytic cell to recognize its target and to adhere to it serves to "focus" the functional responses of the cell along the adherent surface. When stimulated phagocytic cells adhere to basement membranes, the production of reactive oxygen metabolites is greatest along the adherent surface. The fact that this is also the site of greatest lysosomal degranulation indicates a close approximation of the generation and release of these potentially injurious agents. In addition, close approximation of the phagocytic cell with its target serves to exclude macromolecular inhibitors, such as α-1-antiprotease, from the cell–substrate interface. **Thus, in summary, phagocytic cell adherence, secretion of reactive oxygen metabolites, and the release of lysosomal enzymes function in a synergistic manner to enhance cytotoxicity and tissue degradation.**

Chronic Inflammation

Following acute injury of tissue, polymorphonuclear leukocytes are replaced over several days by lymphocytes, mononuclear phagocytic cells, and plasma cells. This "subacute inflammatory response" represents the early stages of resolution leading to the formation of granulation tissue. **Granulation tissue is characterized by the proliferation of endothelial cells and fibroblasts into the area of injury** (Fig. 2-22). Proliferation of endothelial cells leads to the formation of small capillaries and restoration of the vascular supply. The fibroblast restores the connective tissue matrix in the injured tissue by increased synthesis of mucopolysaccharides and collagen types I and III. Proliferation of endothelial cells and fibroblasts is regulated by specific mediators secreted by activated macrophages and T-lymphocytes. Plasma

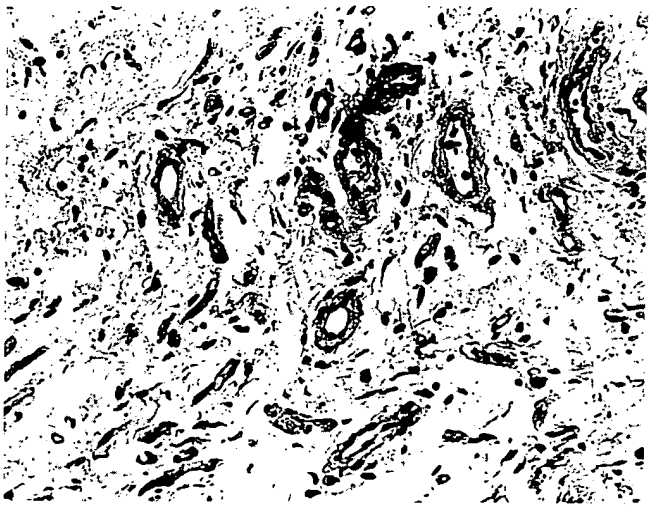
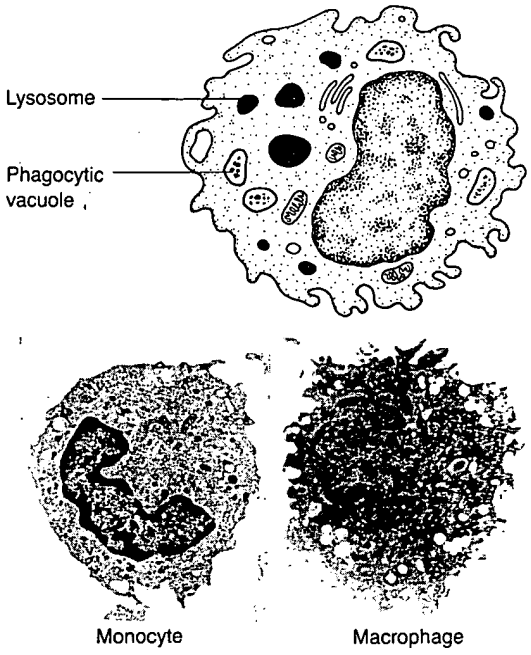


Figure 2-22. Granulation tissue. Chronic inflammatory cells, fibroblasts, loose connective tissue and abundant blood vessels are evident.



CHARACTERISTICS AND FUNCTIONS

- Associated with:
  - –Chronic inflammation
  - –Bacterial phagocytosis
- Regulates lymphocyte responses
  - –Antigen presentation
  - –Monokine production
- Regulates coagulation/fibrinolytic pathway

PRIMARY INFLAMMATORY MEDIATORS

- Lysosomal enzymes
  - –Acid hydrolases
  - –Neutral proteases (e.g., elastase, collagenase, cathepsins)
- Cationic proteins
- Phospholipase A_2
- Prostaglandins, leukotrienes
- Interleukin-1
- Plasminogen activator
- Procoagulant activity
- Oxygen metabolite formation
- Complement activation

Figure 2-23. Monocyte/macrophage: morphology and function.

and platelet-derived factors also regulate endothelial and fibroblast growth. These factors cause an increased proliferation of cells and, in the case of fibroblasts, increased connective tissue synthesis and deposition.

Under conditions in which the inflammatory response is unable to eliminate the injurious agent or restore injured tissue to its normal physiologic state, there may be progression to a state of chronic inflammation. The primary cellular components of the chronic inflammatory response are macrophages, plasma cells, lymphocytes, and, in certain conditions, eosinophils. Chronic inflammation is mediated by both immunologic and nonimmunologic mechanisms and is frequently observed in conjunction with granulation tissue. The macrophage is the pivotal cell in regulating these reactions because it functions as a source of both inflammatory and immunologic mediators (Fig. 2-23). The accumulation of **macrophages** occurs primarily as a consequence of recruitment of circulating monocytes by chemotactic stimuli and their differentiation in tissues (Fig. 2-24). However, the local proliferation and differentiation of resident tissue macrophages may also contribute to the increase in mononuclear phagocytes. As well as generating inflammatory mediators, macrophages regulate lymphocyte responses to antigen and secrete mediators that modulate the proliferation and function of fibroblasts and endothelial cells.

Plasma cells also participate in the chronic inflammatory response (Fig. 2-25). These cells are rich in rough endoplasmic reticulum and are the primary source of antibody production. The accumulation of **lymphocytes** is also a prominent feature of chronic inflammatory reactions (Fig. 2-26) and is important in both humoral and cell-mediated immune responses. **Eosinophils** are occasionally a conspicuous component of the chronic inflammatory response. They are particularly evident during allergic-type reactions and parasitic infections. Eosinophils share many functional features with the neutrophil. Their rhomboid, crystalloid granules are rich in acid phosphatase and have a specific peroxidase activity (Fig. 2-27). The granules also contain a unique eosinophil basic protein that is toxic to certain parasites and normal host cells. However the precise role of eosinophils in chronic inflammatory reactions is less clear. **Polymorphonuclear leukocytes**, although characteristic of acute inflammation, may also be observed at sites of chronic inflammation: **Acute and chronic inflammation represent ends of a dynamic continuum in which the morphologic features of the inflammatory response frequently overlap.**

Granulomatous Inflammation

As we have seen, phagocytosis followed by digestion is the mechanism by which neutrophils inactivate and remove agents that incite an acute inflam-

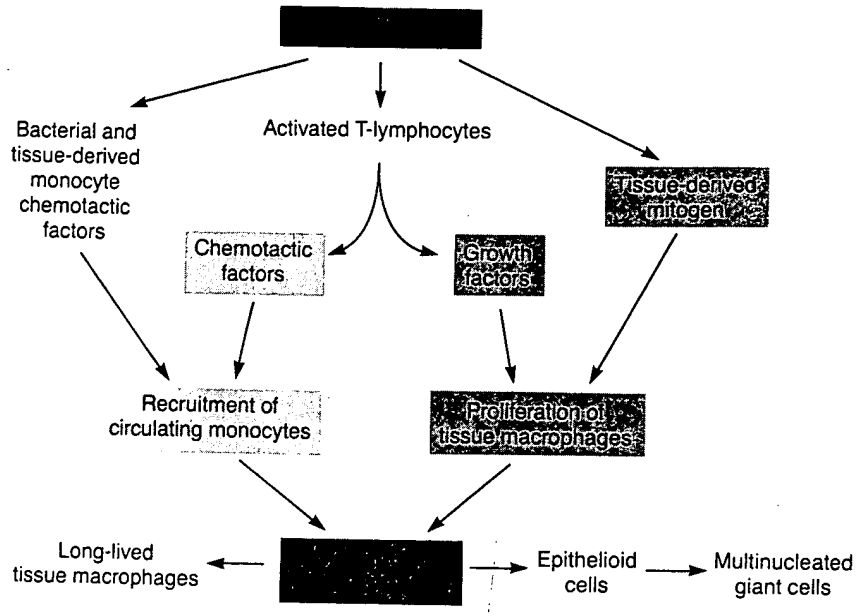


Figure 2-24. The accumulation of macrophages in chronic inflammation.

matory response. However, there are circumstances in which the substances that provoke the acute inflammatory reaction are not digestible by the reacting neutrophils. Such a situation is potentially dangerous because it could lead to a cycle of phagocytosis, failure of digestion, rapid death of the neutrophil, and release of the undigested, provoking agent. The offending material, once free of the neutrophil, would again be phagocytosed by another, newly recruited neutrophil. If the cycle were to continue indefinitely, the result would be persistent and destructive acute inflammation. However, there is a mechanism for dealing with indigestible substances, namely **granulomatous inflammation** (Fig. 2-28).

Granulomatous inflammation is typical of the tissue response elicited by fungal infections, tuberculosis, leprosy, schistosomiasis, and the presence of foreign material (e.g., suture or talc). The principal cells involved in granuloma formation are macrophages and lymphocytes. Macrophages are much longer-lived than neutrophils. If they are not killed by the pathogenic agent that incites the inflammatory reaction, they can store it in their cytoplasm for indefinite periods. Such intracellular sequestration of the noxious agent prevents it from continuing to provoke an acute inflammatory reaction. Normal macrophages are mobile cells that continuously wander through the extravascular connective tissues of the body. Upon phagocytizing and retaining substances that they cannot digest, the macrophages lose their motility and remain in place. A characteristic change in their structure follows that transforms them into so-called **epithelioid cells. Nodular collections of epithelioid cells form the granulomas that are the**

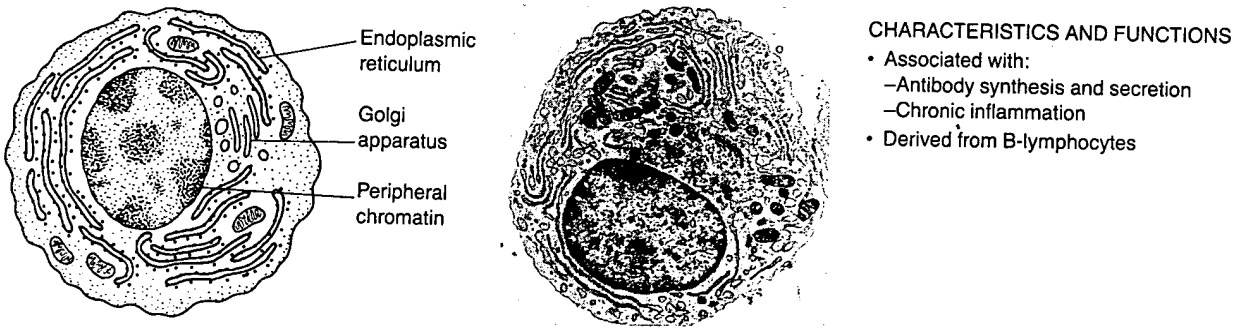
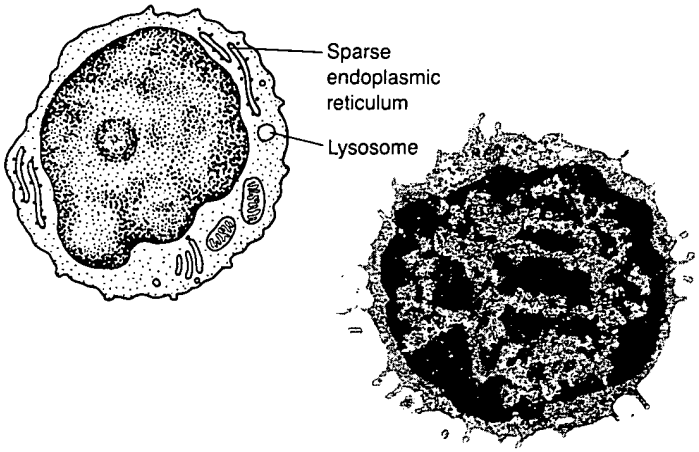


Figure 2-25. Plasma cell: morphology and function.



CHARACTERISTICS AND FUNCTIONS

- Associated with chronic inflammation
- Key cell in humoral and cell-mediated immune responses
- Multiple subtypes:

B cell → Plasma cell → Antibody production

T cell
- Effector cells
  - Delayed hypersensitivity
  - Mixed lymphocyte reactivity
  - Cytotoxic "killer" cells (K-cells)
- Regulatory cells
  - Helper T cells
  - Suppressor T cells

Cytotoxic natural killer (NK) cell

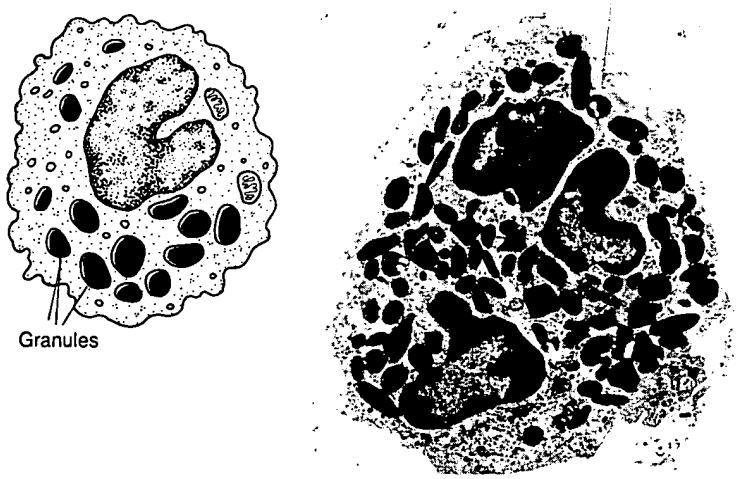
Null cell

Figure 2-26. Lymphocyte: morphology and function.

morphologic hallmark of granulomatous inflammation.

Granulomas are usually small (usually <2 mm), and the collections of epithelioid cells are frequently surrounded by a rim of lymphocytes (Fig. 2-29). Unlike circulating monocytes, epithelioid cells have abundant cytoplasm and numerous lysosomal granules. An additional feature of granulomas is the presence of multinucleated giant cells. These cells are large, contain numerous (up to 40 to 50) nuclei, and are formed from the cytoplasmic fusion of macrophages. When the nuclei are arranged around the periphery of the cell in a horseshoe-shaped pattern, the cell is termed **Langhans' giant cell** (Fig. 2-30). Frequently one can identify a foreign pathogenic agent (e.g., silica or a Histoplasma spore) or other indigestible material within the cytoplasm of a multinucleated giant cell. These cells are referred to as **foreign body giant cells** (Fig. 2-31). Giant cells are functionally inactive, especially when compared to macrophages. In association with the granuloma, one may also see all the other cell types characteristic of chronic inflammation.

Macrophages do have a finite lifespan. A slow turnover of these cells in granulomatous reactions accounts for the continued presence of acute inflammatory cells in granulomas that have been active for months or years. The rate of turnover of the epithelioid cells is also influenced by the toxicity of the inciting agent itself. The more inert the agent, the slower the turnover of the cells. The fate of any granulomatous reaction is influenced not only by the



CHARACTERISTICS AND FUNCTIONS

- Associated with:
  - –Allergic reactions
  - –Parasite-associated inflammatory reactions
  - –Chronic inflammation
- Modulates mast cell-mediated reactions

PRIMARY INFLAMMATORY MEDIATORS

- Reactive oxygen metabolites
- Lysosomal granule enzymes (primary crystalloid granules)
  - –Major basic protein
  - –Eosinophil peroxidase
  - –Acid phosphatase
  - –β-glucuronidase
  - –Arylsulfatase B
  - –Histaminase
- Phospholipase D
- Prostaglandins of E series

Figure 2-27. Eosinophil: morphology and function.

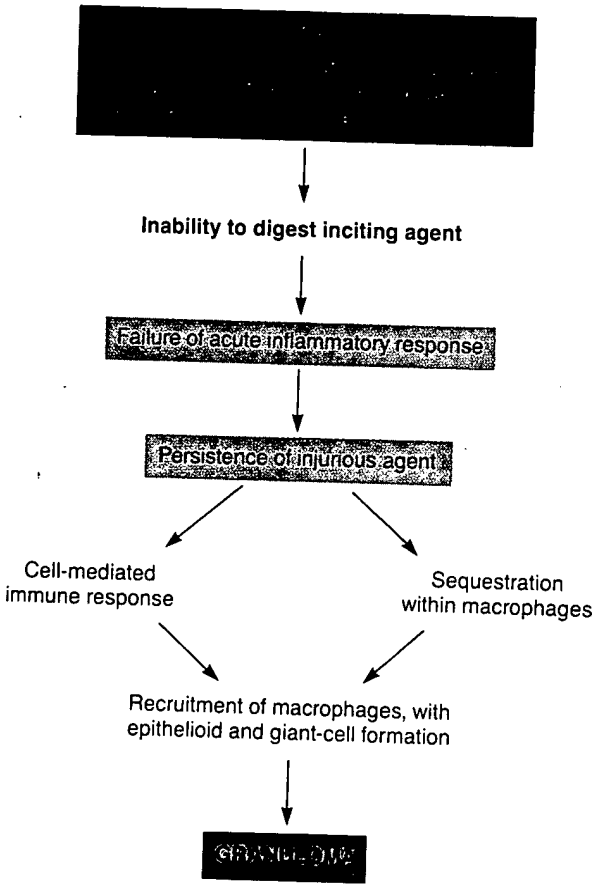


Figure 2-28. Mechanism of granuloma formation.

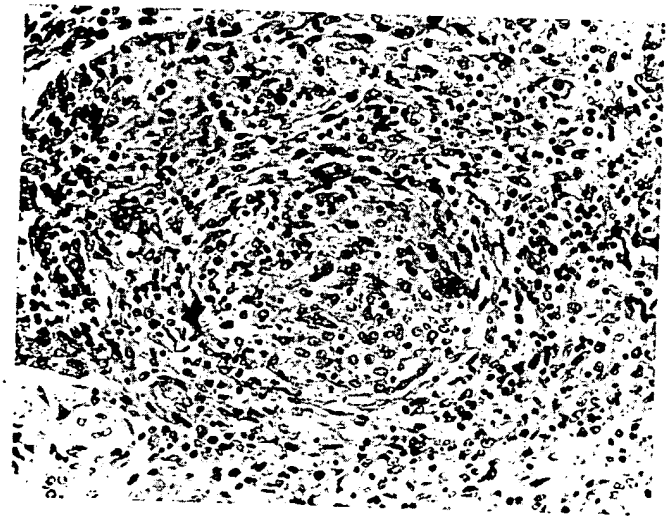


Figure 2-29. Granulomatous inflammation. A granuloma displays epithelioid cells in the center, surrounded by a rim of lymphoid cells. Several multinucleated giant cells are present.

pathogenicity of the inciting agent but also by its immunogenicity. Immunologic sensitivity develops to a persistent inciting agent that is slowly released from the macrophage-epithelioid cell. In particular, cell-mediated immune responses to the inciting agent occur and, in turn, modify the basic granulomatous reaction by recruiting and activating more macrophages and lymphocytes. The contribution of the immune system to the evolution of chronic granulomatous inflammation varies with the immunogenicity of the inciting agent. Figure 2-28 summarizes the mechanisms in the generation of granulomatous inflammation.

Systemic Manifestations of Inflammation

One of the important manifestations of localized inflammatory injury is the subsequent reaction in lymphatics and lymph nodes that drain the tissue. Inflammatory mediators generated at sites of injury, as well as necrotic debris, drain into the lymphatic system and flow to the regional lymph nodes. Under conditions of severe injury there is secondary inflammation of the lymphatic channels (lymphangitis) and the lymph nodes (lymphadenitis). This response represents either a nonspecific reaction to mediators released from the injured tissue or an antigen-specific reaction reflecting an immunologic response to a foreign antigen. Clinically, the inflamed lymphatic channels in the skin manifest as red streaks, and the lymph nodes themselves are enlarged and painful. Lymph nodes can exhibit hyperplasia of the lymphoid follicles (follicular hyperplasia) and proliferation of mononuclear phagocytic cells in the sinuses (sinus histiocytosis).

A clinical hallmark of inflammation is **fever**. Pyrogens are released into the circulation from exogenous sources, such as bacteria, or are produced endogenously. The primary endogenous pyrogen is interleukin-1, a protein secreted primarily by macrophages, which regulates lymphocyte activation during immune responses. This low-molecular-weight protein (15,000 daltons) is released by macrophages following a phagocytic stimulus or exposure to bacterial endotoxin, viruses, or lymphocyte products. Interleukin-1 acts on the thermoregulatory centers within the hypothalamus to initiate the fever response. Interleukin-1 stimulates arachidonic acid metabolism within the hypothalamus, thus leading to prostaglandin synthesis. Inhibitors of cyclo-oxy-

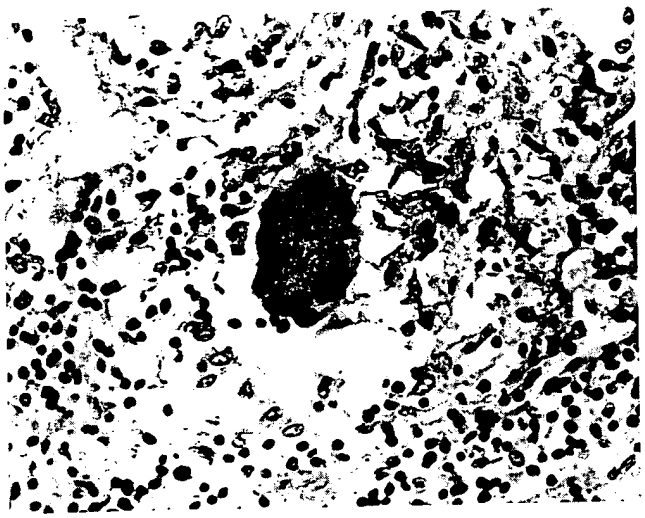


Figure 2-30. Langhans' giant cell. A number of nuclei are arranged on the periphery of an abundant cytoplasm.

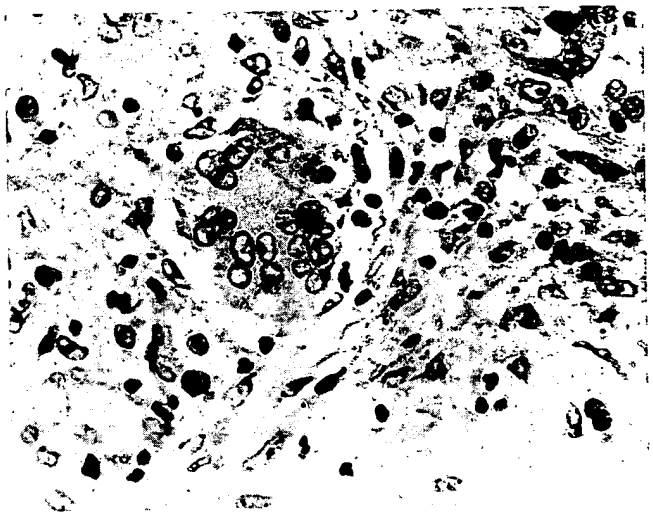


Figure 2-31. Foreign body giant cell. The numerous nuclei are randomly arranged in the cytoplasm.

genase (e.g., aspirin) block the fever response by inhibiting interleukin-1–stimulated PGE_2 synthesis in the hypothalamus.

An additional systemic effect of local inflammation is an increase in the numbers of circulating white blood cells **(leukocytosis).** Leukocytosis is commonly manifested as a two- to threefold increase in the number of white blood cells and reflects principally an increase in circulating polymorphonuclear leukocytes (neutrophilia). In addition, an increase in the number of immature polymorphonuclear leukocytes ("band" forms) is also seen in the peripheral blood. The mechanism for increasing circulating white blood cells involves the release of specific mediators that promote an accelerated release of polymorphonuclear leukocytes from the bone marrow and an increased proliferation of bone marrow precursors. One of the best characterized of the mediators responsible for the increased cell proliferation is a group of compounds referred to as **colony stimulating factors.** These factors are produced by macrophages and T-lymphocytes and stimulate mitotic activity in myeloid precursors in the bone marrow. The circulating levels of the myeloid precursors of leukocytes often may become extremely high, reaching levels of 40,000 to 100,000 cells per mm^3. This extreme elevation in the circulating white blood cell count has been referred to as a **"leukemoid reaction"** and must be differentiated from leukemia. Neutrophilia is most frequently seen in association with bacterial infections and with infarction of tissues. In contrast, viral infections, including infectious mononucleosis, are characterized by an absolute increase in the number of circulating lymphocytes **(lymphocytosis).** During parasitic infections and certain allergic reactions, one may observe an increase in the number of eosinophils in the peripheral blood without an increase in the absolute white blood cell count **(eosinophilia).** Eosinophils, which normally constitute 1% to 3% of peripheral white blood cells, can reach a level of 10% to 15% in eosinophilia.

Under conditions of chronic inflammation, particularly in patients who are nutritionally deprived or who suffer from a chronic debilitating disease such as disseminated cancer, an absolute decrease in circulating white cell counts may be observed **(leukopenia).** The mechanisms responsible for the suppression of leukopoiesis are not well understood. They presumably represent an imbalance in the production of mediators that regulate myeloid and lymphoid precursors in the bone marrow.

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